DRAFT

Addendum

to

ICCVAM Evaluation of *In Vitro* Test Methods for Detecting Potential Endocrine Disruptors:

Estrogen Receptor and Androgen Receptor Binding and Transcriptional Activation Assays

(Proposed Revisions to the List of Recommended Reference Substances for Validation)

14 March 2006

National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods

National Institute of Environmental Health Sciences
National Institutes of Health
U. S. Public Health Service
Department of Health and Human Services

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List of Abbreviations and Acronyms

Antag.	Antagonist
AR	Androgen receptor
BG-1	Ovarian carcinoma derived cells
BRD	Background Review Document
CASRN	Chemical Abstracts Service Registry Number
DDE	1,1-Dichloro-bis[4-chlorophenyl]ethylene
DDT	Dichlorodiphenyltrichloroethane
Dept.	Department
DHT	5α-Dihydrotestosterone
DMSO	Dimethyl sulfoxide
EC_{50}	Half-maximal effective concentration
ED	Endocrine Disruptor
EDSP	Endocrine Disruptor Screening Program
EDSTAC	Endocrine Disruptor Screening and Testing Advisory Committee
EDWG	Endocrine Disruptor Working Group
EPA	U.S. Environmental Protection Agency
ER	Estrogen receptor
FR	U.S. Federal Register
IC_{50}	Concentration of a test substance inhibiting the reference estrogen or androgen response by 50%
ICCVAM	Interagency Coordinating Committee on the Validation of Alternative Methods
JME	Japanese Ministry of the Environment
Ki	Equilibrium dissociation constant of a receptor-ligand
K_{OW}	Octanol/water partition coefficient
LUMI-CELL®	Chemical-activated luciferase expression assay
MeSH	U.S. National Library of Medicine's Medical Subject Headings
Min.	Minimum
mg	Milligram

mL	Milliliter
mM	Millimolar
NICEATM	National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods
NIEHS	National Institute of Environmental Health Sciences
NIH	National Institutes of Health
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
PAH	Polycyclic aromatic hydrocarbon
PCB	Polychlorinated biphenyl
P.L.	Public Law
pM	Picomolar
pmol	Picomole
RBA	Relative binding affinity
RNA	Ribonucleic Acid
TA	Transcriptional activation
μM	Micromolar

PREFACE

In April of 2000, the U.S. Environmental Protection Agency (EPA) asked the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) to evaluate the validation status of *in vitro* estrogen receptor (ER) and androgen receptor (AR) binding and transcriptional activation (TA) test methods, which were proposed as possible components of the EPA Endocrine Disruptor Screening Program (EDSP) (EPA 1998). Because a large number of *in vitro* ER- and AR-based test methods were known to exist, it was expected that at least some of these would have been adequately validated and could, following a review of existing data and verification of their validity, be included in the EDSP. The National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) subsequently compiled available data and information on the *in vitro* ER and AR binding and TA test methods. Four Background Review Documents (BRDs) were produced that provided comprehensive reviews of the available data for each of the four types of test methods.

In collaboration with ICCVAM and the ICCVAM Endocrine Disruptor Working Group (EDWG), NICEATM organized an independent evaluation on May 20-21, 2002 in Research Triangle Park, NC of these *in vitro* test methods for detecting substances with potential endocrine disrupting activity. This meeting was open to the public with time set aside for public comment. A 24-member scientific expert panel (Panel) reviewed the information and recommendations provided in the four draft BRDs and concluded that there were no adequately validated *in vitro* ER- or AR-based test methods. In addition, at the public meeting, the Panel provided recommendations on the following:

- specific test methods that should undergo further evaluation in validation studies and their relative priority for evaluation
- the adequacy of proposed minimum procedural standards
- the adequacy of protocols for specific test methods recommended for validation
- the adequacy and appropriateness of reference substances proposed for validation studies

In October, 2002, NICEATM published:

1) the Panel's report (ICCVAM 2002e)
2) a *Federal Register* (*FR*) notice requesting public comment on the Panel's report (*FR* Vol. 67, No. 204, pp. 64902-64903, October 22, 2002)

ICCVAM considered the Panel's conclusions and recommendations and public comments received in response to the *FR* notice. ICCVAM then developed test method recommendations that included minimum procedural standards and a list of 78 reference substances that should be used to standardize and validate *in vitro* ER and AR binding and TA test methods. ICCVAM's conclusions and recommendations, as well as the final BRDs and other supporting information, were made publicly available in May of 2003 (ICCVAM 2003).

NICEATM recently assessed the commercial availability and cost for the 78 substances recommended by ICCVAM for use in *in vitro* ER and AR binding and TA validation studies. During this assessment, NICEATM identified three substances that are not commercially available, one substance with limited commercial availability, and six substances that were considered relatively expensive. ICCVAM is recommending replacement of those substances that are not commercially available or have limited commercial availability with commercially available substances that have similar ER or AR binding or agonist TA activity profiles, or are similarly concordant for antagonist TA activity across studies. Four of the six more expensive substances were retained because of chemicophysical properties that were considered unique. The two remaining expensive substances are recommended for replacement with less expensive substances that have similar ER or AR binding or agonist TA activity profiles, or that are similarly concordant for antagonist TA activity across studies. These proposed changes to the 78 ICCVAM recommended substances are detailed in this addendum to the report "ICCVAM Evaluation of *In Vitro* Test Methods for Detecting Potential Endocrine Disruptors: Estrogen Receptor and Androgen Receptor Binding and Transcriptional Activation Assays" (ICCVAM 2003).

This updated list of recommended reference substances should facilitate standardization and validation of *in vitro* endocrine disruptor test methods. Data generated from adequately validated and accepted *in vitro* and *in vivo* Tier 1 screening assays will be used to reach weight-of-evidence decisions on whether to conduct large multi-generational Tier 2 *in vivo* studies. It is also anticipated that data obtained during the validation of the four different types of *in vitro* ER- and AR-based test methods will help characterize the extent to which individual or batteries of *in vitro* endocrine disruptor test methods might be used to prioritize substances for Tier 1 testing.

EXECUTIVE SUMMARY

The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) is proposing revisions to the original list of 78 substances recommended for the validation of *in vitro* estrogen receptor (ER) and androgen receptor (AR) binding and transcriptional activation (TA) test methods, based on an assessment of their commercial availability and cost. This assessment determined that three substances are not commercially available and that the commercial availability of another substance is restricted. ICCVAM proposes replacement of those four substances with commercially available substances that have similar ER or AR binding or agonist TA activity profiles, or are similarly concordant for antagonist TA activity across studies. Six substances were identified that were considered relatively expensive. Four of the six expensive substances were retained because their chemicophysical properties were considered unique. The two other substances are recommended for replacement with less expensive substances that have similar ER or AR binding or agonist TA activity profiles, or are similarly concordant for antagonist TA activity across studies.

1.0 INTRODUCTION

The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) compiled a list of 78 substances to facilitate the validation of *in vitro* estrogen receptor (ER) and androgen receptor (AR) binding and transcriptional activation (TA) test methods (ICCVAM 2003). A number of factors and criteria were considered by ICCVAM in compiling this list, including assay data and recommendations provided in Background Review Documents (BRDs) on ER and AR binding and TA test methods (ICCVAM 2002a, b, c, d), and in the ICCVAM Endocrine Disruptor Expert Review Panel Final Report (ICCVAM 2002e). To allow for a direct comparison between results obtained from *in vitro* and *in vivo* ED test methods, the list also includes substances proposed for *in vivo* ED testing by the U.S. Environmental Protection Agency (EPA) and the Organisation for Economic Co-operation and Development (OECD)[1]. These factors and considerations are discussed in detail in the report: *ICCVAM Evaluation of the In Vitro Methods for Detecting Potential Endocrine Disruptors: Estrogen Receptor and Androgen Receptor Binding and Transcriptional Activation Assays* (referred to below as the ICCVAM ED Test Method Evaluation Document) (ICCVAM 2003).

Two practical criteria for reference substances recommended for validation studies are that the substances should be: 1) commercially available, and 2) to the extent possible, reasonably priced. The National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) has recently assessed commercial availability and pricing information for the list of 78 recommended substances and, based on the information obtained, ICCVAM has revised the list based on these criteria. This document:

1. identifies and proposes specific replacements for those substances that are not commercially available, can only be purchased in a limited amount, or are considered

[1] On July 8, 2002, NICEATM received a list of the substances selected or recommended for *in vitro* endocrine disruptor testing by the EPA and for *in vivo* endocrine disruptor testing by the EPA or the OECD from Mr. Gary Timm in the EPA Office of Science Coordination and Policy, Washington, DC. The list was compiled by Mr. James Kariya for presentation at the March 2002 meeting of the EPA ED Methods Validation Subcommittee. On August 4, 2005, NICEATM received an updated list of substances for *in vitro* and *in vivo* endocrine disruptor testing by the EPA from Mr. Timm.

to be relatively expensive (i.e., would cost more that $2000 per 500 mg, the minimum amount that a laboratory might need to test the substance as part of a validation study)

2. reviews the criteria used to select the original substances for *in vitro* ER and AR binding and TA validation studies, which were also used by ICCVAM in selecting the proposed replacements

2.0 BASIS FOR SELECTION OF THE ORIGINAL ICCVAM RECOMMENDED REFERENCE SUBSTANCES FOR VALIDATION OF *IN VITRO* ENDOCRINE DISRUPTOR TEST METHODS

2.1 Background Review Documents

In February 2002, four draft BRDs were published that documented available data for ER and AR binding and TA test methods for detecting endocrine disruptors:

- Current Status of Test Methods for Detecting Endocrine Disruptors: *In Vitro* Estrogen Receptor Binding Assays (ICCVAM 2002a) (referred to below as the ER Binding BRD)
- Current Status of Test Methods for Detecting Endocrine Disruptors: *In Vitro* Estrogen Receptor Transcriptional Activation Assays (ICCVAM 2002b) (referred to below as the ER TA BRD)
- Current Status of Test Methods for Detecting Endocrine Disruptors: *In Vitro* Androgen Receptor Binding Assays (ICCVAM 2002c) (referred to below as the AR Binding BRD)
- Current Status of Test Methods for Detecting Endocrine Disruptors: *In Vitro* Androgen Receptor Transcriptional Activation Assays (ICCVAM 2002d) (referred to below as the AR TA BRD)

Each draft BRD included a list of proposed reference substances recommended for future validation studies of the test method considered. Selection of these substances was based on:

- the availability of published or submitted data demonstrating reproducible positive or negative responses in multiple studies and/or test methods

- the extent to which these substances covered the range of negative to weakly positive to strongly positive responses
- the distribution of the proposed substances among chemical classes

2.2 ICCVAM Endocrine Disruptor Expert Panel Recommendations

An Expert Panel (Panel) met in June 2002 and developed recommendations on the adequacy and appropriateness of the substances proposed in the draft BRDs for use in future validation studies. The Panel generally agreed with the lists of proposed substances but also recommended that:

- for a specific receptor (ER or AR), the same substances should be tested in binding and TA agonism and antagonism test methods
- the proportion of negative substances in each list should be increased to at least 25% of the total number of substances to better evaluate test method specificity
- substances (e.g., actinomycin D, cycloheximide, sodium azide, 12-O-tetradecanoylphorbol-13-acetate) that might interfere indirectly with reporter gene transcriptional activation by altering metabolic pathways, such as RNA and protein synthesis, should be included
- additional substances from underrepresented chemical classes (e.g., phthalates, polycyclic aromatic hydrocarbons [PAHs], polychlorinated biphenyls) should be included

2.3 Development of the Original ICCVAM Recommended Reference Substances

In late 2002, ICCVAM reviewed the Panel's recommendations regarding substances that should be used in future validation studies and developed a final list of recommended reference substances. To meet the Panel's recommendation that at least 25% of the substances proposed for validation studies be negative for binding or TA for the receptor being used, an assumption was made that substances positive in ER binding or TA test methods would likely be negative in the corresponding AR-based test methods and vice versa, and that such substances could serve as presumptive negatives in the alternative

receptor-based test methods. This approach would also minimize the total number of different substances that would be needed to validate ER/AR test methods.

2.3.1 Selection of the Original Candidate Substances

Initially, 122 substances were identified as candidate reference substances for validation studies (**Appendix A**). This list of candidates consisted of:

- 85 substances recommended in the four draft BRDs for future validation studies (Section 12.0, Table 12-1 in the ER and AR Binding Assay BRDs, and Section 12.0, Tables 12−1 and 12−2 in the ER and AR TA BRDs)
- 44 substances scheduled for testing in *in vivo* mammalian endocrine disruptor test methods by the EPA and the OECD; 22 of which were included in the lists mentioned above. Adding the remaining 22 substances increased the total number of candidate substances to 107. The *in vivo* list included five substances (oxazepam, phenobarbital, L-thyroxine, ammonium perchlorate, and propylthiouracil) that are known to disrupt thyroid function *in vivo* and thus could likely serve as presumed negative substances in *in vitro* ER and AR binding and TA validation studies.
- 38 substances were scheduled for testing in *in vitro* endocrine disruptor test methods by the EPA, 29 of which were included in the BRD lists above. Adding the additional nine substances increased the total number of candidate substances to 116.
- The Panel specifically recommended six additional substances (actinomycin D, bicalutamide, cycloheximide, hydroxyflutamide, sodium azide, and 12-O-tetradecanoylphorbol-13-acetate), resulting in a total of 122 candidate substances.

Five of the candidate substances (butylbenzyl phthalate, diethylhexyl phthalate, dibenzo[*a,h*]anthracene, fluoranthene, and zearalenone) belong to chemical classes that had been underrepresented in the BRD lists (phthalates for the first two substances, PAHs for the second two substances, and resorcylic acid lactone/phenol for the last substance). In addition, seven of the candidate substances (bisphenol A, 1,1-dichloro-bis[4chlorophenyl] ethylene, dichlorodiphenyltrichloroethane, di-(2-ethylhexyl)phthalate, di-n-butylphthalate, nonylphenol, and octylphenol) have been tested *in vivo* for endocrine disruptor activity by the Japanese Ministry of the Environment (JME).

2.3.2 Selection of the Original 78 Recommended Substances

The list of 122 candidate substances was reduced to 114 candidates based on the following:

- methyl parathion and 2,3,7,8 tetrachlorodibenzo-p-dioxin, highly toxic substances proposed by the EPA for *in vivo* testing, were excluded to avoid potential worker exposure
- 4-chloro-4'-biphenylol and 2',4',6' trichloro-4-biphenylyol, two substances recommended in the draft BRDs, and Arochlor 1254, a substance proposed for *in vivo* testing by the EPA, were excluded because of hazardous waste disposal concerns
- letrozole was excluded because EPA was not sure that it would be tested *in vivo* and because of the absence of *in vitro* data
- testosterone propionate, also proposed for *in vivo* testing by EPA, was excluded because it is readily hydrolyzed *in vivo* to its parent compound, testosterone, which has been tested much more extensively in multiple *in vitro* endocrine disruptor test methods
- tamoxifen citrate, proposed by the EPA for *in vitro* testing, was excluded because its parent compound, tamoxifen, has been tested much more extensively in multiple *in vitro* endocrine disruptor test methods

The remaining list of 114 candidate substances was reduced to 78 substances (**Appendix A**) by excluding substances not scheduled for *in vitro* testing by the EPA or for *in vivo* testing by EPA and OECD (with the exceptions noted above). Thus, 39 of the 44 substances proposed for *in vivo* testing by EPA and OECD are included in this list, as well as 37 of the 38 substances proposed for *in vitro* testing by the EPA. The number of substances recommended in the draft BRDs for the validation of *in vitro* ER and AR binding and TA assays and their expected performance in the various *in vitro* endocrine disruptor test methods are provided in **Table 1**. **Table 2** contains the expected performance for *in vitro* ER-based test methods while **Table 3** contains similar information for *in vitro* AR-based test methods.

Table 1 Numbers of Substances Recommended in the Draft BRDs for the Validation of *In Vitro* ER and AR Binding and TA Assays[a]

In Vitro Assay Type	Number of Substances	Number of Positive Substances	Number of Negative Substances
ER Binding	33	30 (91%)	3 (9%)
ER TA Agonist	31	26 (84%)	5 (16%)
ER TA Antagonist	21	17 (81%)	4 (19%)
AR Binding	31	28 (90%)	3 (10%)
AR TA Agonist	28	18 (64%)	10 (36%)
AR TA Antagonist	25	21 (84%)	4 (16%)

[a]Based on information provided in BRDs on ER and AR binding and TA test methods (ICCVAM 2002a,b,c,d).

Table 2 Distribution of Anticipated Responses of the Recommended Test Substances in *In Vitro* ER Binding and TA Assays[a]

Expected Response	ER Binding	ER TA	
		Agonist	Antagonist
Positive[b] and Presumed Positive[c]	41 (53%)	35 (45%)	11 (14%)
Negative[d] and Presumed Negative[e]	37 (47%)	43 (55%)	67 (86%)
Total	78	78	78

[a]Based on information provided in Sections 3.0 through 6.0 of the ICCVAM ED Test Method Evaluation Document**.** Counts include the recommended reference estrogen, 17β-estradiol.

[b] Represents substances for which ER binding or TA data are available, which indicate a positive response in the respective test method (i.e., substances tested in more than one study that were positive in > 50% of the studies).

[c] Represents substances that were positive in ≤ 50% of reported studies; that were positive but tested in only one study; or that have no relevant receptor binding or TA data available for the respective test method but which are presumed positive based on their known mechanism of action or their responses in other endocrine disruptor screening test methods (e.g., methyl testosterone, an ER agonist, is presumed positive in ER binding assays).

[d] Represents substances that tested negative for ER binding or ER TA in multiple studies, when tested up to the limit dose

[e] Represents substances which are presumed negative based on the available data, their known mechanism of action, or their responses in other endocrine disruptor screening test methods (e.g., anastrazole and fadrozole, known aromatase inhibitors, are presumed negative in ER binding and TA assays).

Table 3 Distribution of Anticipated Responses of the Recommended Test Substances in *In Vitro* AR Binding and TA Assays[a]

Expected Response	AR Binding	AR TA	
		Agonist	Antagonist
Positive[b] and Presumed Positive[c]	34 (44%)	22 (28%)	21 (27%)
Negative[d]	44 (56%)	56 (72%)	57 (73%)
Total	78	78	78

[a] Based on information provided in Sections 3.0 through 6.0 of the ICCVAM ED Test Method Evaluation Document**.** Counts include the recommended reference androgen, methyltrienolone.

[b] Represents substances for which receptor binding or TA data are available, which indicate a positive response in the respective test method (i.e., substances tested in more than one study that were positive in > 50% of the studies).

[c] Represents substances that were positive in ≤ 50% of reported studies; that were positive but tested in only one study; or that have no relevant receptor binding or TA data available for the respective test method but which are presumed positive based on their known mechanism of action or their responses in other endocrine disruptor screening test methods (e.g., ketoconazole, an AR agonist, is presumed positive in AR binding assays).

[d] Represents substances that tested negative but had not been tested in multiple AR binding or in multiple AR TA studies up to the limit dose (i.e., 1 mM); or that have no relevant receptor binding or TA data available for the test method of interest but which are presumed negative based on their known mechanism of action or their responses in other endocrine disruptor screening assays (e.g., anastrozole and fadrozole, known aromatase inhibitors, are presumed negative in AR binding and TA assays). No substances could be classified as negative for AR binding or AR TA since none had been tested in multiple studies at or above the recommended limit dose of 1 mM.

Based on the available data, approximately 47% and 56% of the substances are expected to be negative in *in vitro* ER- and AR-based test methods, respectively.

2.3.3 Purpose and Advantages of the Original List of 78 Substances

The purpose of the list of 78 substances is to ensure that the comparative reliability and performance of *in vitro* ER and AR binding and TA test methods are adequately characterized across a broad range of chemical classes and responses. The current goal of the EPA is to validate *in vitro* ER and AR binding and TA test methods as components of the Endocrine Disruptor Screening Program Tier 1 screening battery, which includes both *in vitro* and *in vivo* test methods. This list includes most of the substances proposed for the validation of Tier 1 and Tier 2 *in vivo* test methods, which will help characterize the usefulness and limitations of the Tier 1 screening battery for prioritizing substances for Tier 2 testing, and hopefully facilitate development of more predictive *in vitro* endocrine disruptor test methods. The current proportion of negative and presumed negative substances in this

list is greater than the 25% recommended by the Expert Panel. However, for most of the negative substances, the classification of negative is not based on actual data, and, despite expectations to the contrary, a number of substances expected to be discordant for activity between ER- and AR-based test methods have been reported as active in both.

2.3.4 Original Minimum Lists of Substances for Validation of *In Vitro* Endocrine Disruptor Test Methods

Because the purpose of these *in vitro* test methods in the Tier 1 screening battery is to provide binding and TA data that will be considered in a weight-of-evidence evaluation to prioritize substances for Tier 2 testing, characterizing the activity of all of the substances expected to be negative *in vitro* (e.g., thyroid disruptors, aromatase inhibitors) may not be essential. Thus, ICCVAM developed minimum lists of substances that should be given priority during the validation of *in vitro* ER and AR binding and TA test methods. For each receptor type, the same substances are proposed for testing in binding and TA (agonist and antagonist) studies. This approach will allow for a direct comparison of the reliability and performance of these different types of *in vitro* endocrine disruptor test methods. The substances proposed in the draft BRDs and those being tested by the EPA in *in vitro* studies have been used as the foundation for each minimum list. The additional substances recommended by the Panel and those likely to be negative for the endpoint being assessed, complete the list. The minimum lists contain 53 substances for ER binding and TA studies and 44 substances for AR binding and TA studies, with similar distributions of substances across the ranges of responsiveness and chemical classes as contained in the list of 78 substances. The original 53 and 44 substances selected for the minimum lists are provided in **Appendix A**.

2.3.5 Data Supporting the Original Recommended Substances

Of the 78 substances included in the original list, relevant quantitative data from *in vitro* ER and AR binding and TA studies was not available for all substances. The number and percentage of the total list of substances for which ER and AR quantitative binding and quantitative or qualitative TA data were available are presented in **Table 4**.

Table 4 Number of Substances from Total List of 78 for AR and ER Binding and TA Test Methods for Which Relevant Quantitative or Qualitative Data was Identified[a]

	AR Binding	AR-TA		ER Binding	ER-TA	
		Agonist	Antagonist		Agonist	Antagonist
Number of Substances	33[b]	45[c]	27[c]	45[b]	45[c]	18[c]
Percentage of Total	42%	58%	35%	58%	58%	23%

[a] Based on information provided in Sections 3.0 through 6.0 of the ICCVAM ED Test Method Evaluation Document.

[b] The number of substances for which relevant quantitative data for *in vitro* binding studies was available.

[c] The number of substances for which relevant quantitative or qualitative data from agonist and antagonist studies was available.

Many of these substances were tested in only one or two of the four types of test methods and often once only. Thus, there are numerous data gaps, as well as incomplete information, regarding how the different types of *in vitro* ER- and AR-based test methods will respond to the 78 recommended substances. Because the data were generated by studies conducted by different laboratories using different experimental protocols, the data are highly variable and, thus, should not be used as definitive target values to be obtained during future validation studies. ICCVAM intends to update the database for the revised list of recommended substances. Accordingly, a *FR* request for additional data will be published.

3.0 THE REVISED ICCVAM REFERENCE SUBSTANCE LIST FOR THE VALIDATION OF *IN VITRO* ESTROGEN RECEPTOR AND ANDROGEN RECEPTOR BINDING AND TRANSCRIPTIONAL ACTIVATION TEST METHODS

NICEATM has assessed commercial availability for the complete original list of 78 recommended substances. This assessment indicated that anastrozole, CGS18320B, and fadrozole are not commercially available and that the commercial availability of ICI 182,780 continues to be restricted to the purchase of 100 mg/year/institution.

NICEATM has also assessed current costs for the remaining 74 substances. The practical consideration of reasonable pricing was based on the price for 500 mg of substance[2], the expected minimum amount required per laboratory to conduct an *in vitro* ER or AR binding or TA validation study. The cost per 500 mg of substance ranged from $7.80 to $15,500 and all but six of the 74 substances were priced at less than $2000. Therefore, "reasonable pricing" for the minimum amount of substance required to conduct an *in vitro* ER or AR binding or TA validation study has been defined as a substance costing less than $2,000 per laboratory. Based on this definition, actinomycin D ($2,285), zearalenone ($2,760), hydroxyflutamide ($2,940), 4-hydroxytamoxifen ($5,270), 12-*O*-tetradecanoylphorbol-13-acetate ($11,220), and methyltrienolone ($15,500) do not meet reasonable pricing criteria. Actinomycin D is being retained as a reference substance despite its cost as it is the only RNA synthesis inhibitor (Gorski et al. 1975; Kersten and Kersten 1974; Villee et al. 1975) on the current list of 78 reference substances and it was listed as an original minimum substance to be tested for both ER and AR validation studies. Hydroxyflutamide is also being retained as an ED reference substance because:

1) it was specifically recommended by the Panel
2) its AR activity is well documented in the scientific literature
3) it was originally listed as a minimum substance to be tested for both ER and AR validation studies.

12-*O*-tetradecanoylphorbol-13-acetate is being retained because:

1) it is the only phorbol ester on the list of 78 recommended substances
2) it has known mitogenic activity that is not mediated via an ER-dependent pathway (Bamberger et al. 1998; Darne et al. 1998; Gagne et al. 1994; Martin et al. 1995; Whitman et al. 1989)
3) it was originally listed as a minimum substance to be tested for both ER and AR validation studies.

[2] 500 mg was determined to be a sufficient amount of chemical per laboratory for agonist and antagonist studies, with triplicate wells run on three separate occasions following a range-finder test, and assuming that testing will be conducted to a limit dose of 1 mM.

4-hydroxytamoxifen is being retained because:

1. it is the active metabolite of tamoxifen and is therefore active in all cell based systems
2. it is well represented in the scientific literature
3. it was originally listed as a minimum substance to be tested for both ER and AR validation studies

The proposed replacements for the six substances that are not currently commercially available, are available only in limited quantities, or are classified as relatively expensive (with the exceptions noted) were chosen based primarily on the similarity of ER or AR binding or agonist TA activity profiles, or on similar concordance for antagonist TA activity across studies to those reference substances recommended for replacement. Activity profiles for substances were either derived from quantitative ER and AR relative binding affinity (RBA) data, or from quantitative ER and AR TA agonist half maximal effective dose (EC_{50}) data.

Substances were classified for binding as follows:

- strongly active (RBA value was >1, designated as +++)
- moderately active (RBA value was between 1 and 0.01, designated as ++)
- weakly active (RBA value was < than 0.01, and designated as +)
- negative (designated as -) when an RBA value could not be determined

Substances were classified for ER and AR TA agonism as follows:

- strongly active (EC_{50} value was <0.001 μM, designated by +++)
- moderately active (EC_{50} value was between 0.001 and 0.1 μM, designated by ++)
- weakly active (EC_{50} value was >0.1 μM, designated by +)
- negative (designated by -) when no agonist activity could be detected

Due to a lack of quantitative ER and AR TA antagonist activity data, substances were classified for ER and AR TA antagonism as follows:

- uniformly active in multiple assays (designated as ###)
- active in the majority of assays in which it was tested (designated as ##)

- active in the single assay in which it was tested (designated as #)
- found uniformly negative in all assays in which it was tested (designated as -)

Secondary considerations for potential replacements were for substances classified as "Substances Considered but not Included for Validation" from the original list of 122 or for substances being evaluated for ED activity by the EPA. A review of the current scientific literature was also conducted to identify potential replacements for substances for which a good fit for activity could not be made with substances from the original list of 122 or the substances being tested by the EPA.

The six ED reference substances recommended for replacement and their proposed replacements include (see also **Tables 5** and **6**):

- Three of the six substances recommended for replacement are aromatase inhibitors (anastrozole, CGS 18320B, and fadrozole) with presumed negative ER and AR activity profiles. The proposed replacements for these three substances are also aromatase inhibitors (4-OH androstenedione, chrysin, and dicofol) and are also presumed to have negative ER and AR activity profiles. 4-OH androstendione and dicofol are classified as "Substances Considered but not Included for Validation" on the original list of 122 substances. Chrysin and dicofol are being evaluated for ED activity by the EPA.
- The proposed replacement for ICI 182,780 as the reference standard for ER TA antagonist test methods is raloxifene, a "Substance Considered but not Included for Validation" on the original list of 122 substances. Although raloxifene may act as an agonist in some *in vitro* systems, it is also classified as a potent Type 1 partial ER antagonist (MacGregor and Jordan 1998) with IC_{50} values in the nanomolar range in a variety of ER TA and cell replication test methods. Although ICI 182,780 is classified as a Type 2 pure antagonist (binding prevents nuclear uptake of ER) (MacGregor and Jordan 1998), the compound is only a marginally more potent antagonist than raloxifene in similar ER TA and cell replication test methods with

Table 5 ED Reference Substances that are Not Commercially Available versus Proposed Replacement Substances

Status	*Substance	Action	Original Min.[a] List for ER	Original Min.[a] List for AR	EPA/ OECD *In Vivo* Testing[b]	ER Binding Activity[c]	ER Agonist Activity[d]	ER Antag.[e,f]	AR Binding Activity[c]	AR Agonist Activity[d]	AR Antag.[e,f]	Total Cost Per 500 mg
Original	Anastrozole	Aromatase Inhibitor			IM					-		Not Commercially Available
Replacement	*4-OH Androstenedione	Aromatase Inhibitor			AROM	+	-		+++			$53
Original	CGS 18320B	Aromatase Inhibitor			407							Not Commercially Available
Replacement	Chrysin	Aromatase Inhibitor			AROM							$60
Original	Fadrozole	Aromatase Inhibitor	yes	yes	F-PA; FRS; IM							Not Commercially Available
Replacement	*Dicofol	Aromatase Inhibitor			AROM		+	-				$88
Original	ICI 182,780	ER Antagonist	yes		IM	+++	-	###				Limited to 100 mg/yr
Replacement	*Raloxifene HCl[f]	ER Antagonist				+++	-	###				$235

* on original ICCVAM list of 122.

[a] Min. = Minimum

[b] 407 = 407 protocol of the Uterotrophic Assay. In the Uterotrophic Assay, the weight of the uterus is determined after exposure of the rat or mouse to the test substance for three days. In the Hershberger assay, sex accessory gland weights are determined in castrated male rats 4-7 days after treatment of the animals with the test substance (agonistic response) or the test substance with testosterone (antagonistic response). The 407 protocol assesses the effect on all organs including the reproductive organs, of varying concentrations of the test substance administered daily to seven week-old female rats for 28 days. After treatment, the estrus cycle is evaluated by daily vaginal smears for 5 days while treatment is continued; AROM = The EPA Placental Aromatase Assay; F-PA = Female Pubertal Assay. In the female (F), the Pubertal Assay (F-PA) measures the time it takes for the vaginal opening to be observed following single or multiple daily treatments from 21 days of age (weaning); FRS = Fish Reproductive Screen; IM = The Intact Male Assay. In the Intact male assay (IM), adult male rats (70-90 days of age) are dosed daily by intraperitoneal injection for 14 days and sacrificed 24 hours after the last dose. The testes, epididymes, seminal vesicles, and prostate are weighed. One cauda epididymis is weighed and the sperm found in this cauda are evaluated for motility and concentration. One testis, epididymis, and thyroid gland are fixed for histological evaluation. Blood hormone levels are measured. This assay detects effects on male reproductive organs that are sensitive to antiandrogens and substances that interfere with testosterone biosynthesis.

626 [c]+++ Indicates that the substance was strongly active as measured by the relative binding affinity (RBA) (RBA value was >1); ++ indicates that the substance was
627 moderately active (RBA value was between 1 and 0.01); + indicates that the substance was weakly active (RBA value was < than 0.01); - indicates that an RBA
628 value could not be determined.
629 [d]+++ Indicates that the substance was strongly active (half maximal effective dose [EC_{50}] value was <0.001 µM); ++ indicates that the substance was moderately
630 active (EC_{50} value was between 0.001 and 0.1 µM: + indicates that the substance was weakly active (EC_{50} value was >0.1 µM).
631 [e] Antag. is Antagonist
632 [f]### indicates that the substance was uniformly positive in multiple assays; ## indicates that the substance was positive in the majority of assays in which it was
633 tested; # indicates that the substance was positive in the single assay in which it was tested; - indicates that the substance was uniformly negative in all assays.
634 [g] Note, Raloxifene may act as an agonist in some *in vitro* systems.
635

Table 6 ED Reference Substances Where Total Cost Per Laboratory is in Excess of $2000 versus Proposed Replacement Substances

Status	*Substance	Action	Original Min.[a] List for ER	Original Min.[a] List for AR	EPA/ OECD *In Vivo* Testing[b]	ER Binding Activity[c]	ER Agonist Activity[d]	ER Antag.[e,f]	AR Binding Activity[c]	AR Agonist Activity[d]	AR Antag.[e,f]	Total Cost Per 500 mg
Original	Methyltrienolone	AR Agonist		yes			-		+++	+++		$15,500
Replacement	*19-Nortestosterone	AR Agonist				++	+\-		+++	+++		$90
Original	Zearalenone	ER Agonist.	yes			+++	++	#		-		$2,760
Replacement	Resveratrol	ER Agonist.				+	++	#			#	$226

* on original ICCVAM list of 122.

[a] Min. = Minimum

[b] None of these substances have been indicated as being evaluated for ED activity by the EPA or OECD.

[c] +++ Indicates that the substance was strongly active as measured by the relative binding affinity (RBA) (RBA value was >1); ++ indicates that the substance was moderately active (RBA value was between 1 and 0.01); + indicates that the substance was weakly active (RBA value was < than 0.01); - indicates that RBA value could not be determined; ± indicates an equivocal response (i.e., in different studies, the substance was reported as positive and negative).

[d] +++ Indicates that the substance was strongly active (half maximal effective concentration [EC_{50}] value was <0.001 µM); ++ indicates that the substance was moderately active (EC_{50} value was between 0.001 and 0.1µM: + indicates that the substance was weakly active (EC_{50} value was >0.1 µM); +/- indicates that the substance was weakly active or negative in different assays.

[e] Antag. is Antagonist

[f] ### indicates that the substance was uniformly positive in multiple assays; ## indicates that the substance was positive in the majority of studies in which it was tested; (#) indicates that the substance was positive in the single assay in which it was tested;
- indicates that the substance was uniformly negative in all assays.

- IC_{50} values in the high picomolar to low nanomolar range (Maggiolini et al. 2004; Stygar et al. 2003; Wijayaratne et al. 1999; Wilson et al. 2004; Yamamoto et al. 2005). When raloxifene is included with a weak antagonist (e.g., flavone, which is four orders of magnitude less potent than raloxifene) as positive controls, the range of potential ER activity is well bracketed by controls.
- The proposed replacement for methyltrienolone is 19-nortestosterone. Both substances are relatively active (EC_{50} values are <0.001 μM) and 19-nortestosterone is classified as a "Substance Considered but not Included for Validation" on the original list of 122 substances.
- The proposed replacement for zearalenone is resveratrol. Both substances are moderately active (EC_{50} values are between 0.001 μM and 0.1 μM) for ER agonism. The ER activity for resveratrol was acquired through a search of the scientific literature (Bhat et al. 2001; Gao et al. 2004; Klinge et al. 2005; Li et al. 2004).

Substances being recommended for replacement are indicated by bolded text in **Appendix A** and their proposed replacements are indicated in bolded and italicized text.

The proposed ICCVAM revised list of reference substances is provided in **Appendices B-1** through **B-6.** The proposed revised lists are sorted by ER binding activity (**Appendix B-1**), ER TA agonist activity (**Appendix B-2**), ER TA antagonism (**Appendix B-3**), AR binding activity (**Appendix B-4**), AR TA agonist activity (**Appendix B-5**), and AR TA antagonism (**Appendix B-6**) for each recommended substance. Also listed in each appendix is relevant information about each substance (e.g., chemical class, chemical/physical properties, cost).

4.0 REVISED CHEMICAL CLASS INFORMATION FOR THE ICCVAM REFERENCE SUBSTANCE LIST

The chemical classes assigned to each reference substance in the original list (ICCVAM 2003) were revised based on a chemical classification system consistent with the U.S. National Library of Medicine's Medical Subject Headings (MeSH; Available: http://www.nlm.nih.gov/mesh), an internationally recognized standardized classification

scheme. This scheme was used to ensure consistency in classifying substances by chemical class among all test methods considered by ICCVAM. For ER binding and TA test methods, the distribution of substances by chemical class, as well as the distribution within each chemical class by relative activity for ER and AR binding, agonist TA activity profiles, and concordance for antagonist TA activity across studies are provided for all 78 reference substances in **Table 7** and for the minimum list of 53 substances in **Table 8**. The corresponding information for AR binding and TA test methods are provided for all 78 reference substances in **Table 9** and for the minimum list of 44 substances in **Table 10**.

5.0 SUMMARY

ICCVAM is recommending revisions to the 78 original substances recommended for use in future *in vitro* ER/AR binding and TA validation studies based on commercial availability and cost. This assessment indicated that three substances (anastrozole, CGS18320B, and fadrozole) are not commercially available and that the availability of ICI 182,780 is restricted. The assessment also indicated that six substances cost more than $2000 for 500 mg, the expected minimum amount of substance required per laboratory to conduct an ED validation study. "Reasonable pricing" has been defined as substances costing less than $2000 per laboratory for a validation study. Based on this definition, actinomycin D, zearalenone, hydroxyflutamide, 4-hydroxytamoxifen, 12-*O*-tetradecanoylphorbol-13-acetate, and methyltrienolone do not meet reasonable pricing criteria. Actinomycin D has been retained despite its cost as it is the only RNA synthesis inhibitor on the current list of 78 reference substances and because it is listed as a minimum substance to be tested for both ER and AR validation studies. Hydroxyflutamide will be retained as an ED reference substance because it was specifically recommended by the Panel, its AR activity is well documented in the scientific literature, and it is listed as a minimum substance to be tested for both ER and AR validation studies. 12-*O*-tetradecanoylphorbol-13-acetate will be retained because it is the only phorbol ester on the list of 78 recommended substances, it has known mitogenic activity that is not mediated via the ER pathway, and it is listed as a minimum substance to be tested for both ER and AR validation studies. 4-hydroxytamoxifen will be retained

Table 7 Revised ICCVAM List of 78 Reference Substances For *In Vitro* Estrogenic Receptor Binding and Transcriptional Activation Validation Studies – Distribution of Substances by Chemical Class and Available Activity Data

MeSH[4] Chemical Classes	Distribution of Substances[5]	Binding[1]					Agonism[2]					Antagonism[3]				
		+++	++	+	+-	-	+++	++	+	+-	-	###	##	#	#-	-
Amides	3				1						1					
Amines	2		1													
Amino Acids	1					P[6]					P					P
Azides	1					P					P					P
Carboxylic Acids	5		1	1	1	1		1	1							1
Esters	1				1				1							1
Flavonoids	9	2	3	1		1	1	1	3	2		2		1	1	3
Heterocyclic Compounds	23	3	4	2	1	1	1	1	4	2	3	2		2	1	4
Hydrocarbons (Cyclic)	6	3		1			1	1	1	1	1	2		1		2
Hydrocarbons (Halogenated)	5		2	1	1				5						1	3
Imidazoles	1					P					P					P
Indoles	1					P					P					P
Ketones	1					P					P					P
Lactones	1					P					P					P
Onium Compounds	1					P					P					P
Phenols	8		4	1				3	1					1		1
Phthalic Acids	3				2	1		1	1	1						2
Polycyclic Compounds	4					2					3		1			1
Pyrimindines	3									1	1				1	
Salts (Inorganic)	1					P					P					P
Steroids	22	5	3	3	1	2	4	3		4	7					5
Ureas	1										1					
Totals[7]	**102**	**13**	**18**	**10**	**8**	**17**	**7**	**11**	**17**	**11**	**26**	**6**	**1**	**5**	**4**	**34**

[1] +++ Indicates that the substance was strongly active as measured by relative binding affinity (RBA) compared to estradiol (RBA value was >1); ++ indicates that the substance was moderately active (RBA value was between 1 and 0.01); + indicates that the substance was weakly active (RBA value was < than 0.01); - indicates that an IC_{50} value was not obtained and thus an RBA value could not be determined; ± indicates an equivocal response (i.e., in different studies, the substance was reported as positive and negative). The inhibitory concentration 50 (IC_{50}) is the concentration of test substances that displaces 50% of the radiolabeled reference estrogen from the receptor.

[2] +++ Indicates that the substance was strongly active (the EC_{50} value was <0.001 µM); ++ indicates that the substance was moderately active (EC_{50} value was between 0.001 and 0.1 µM); + indicates that the substance was weakly active (EC_{50} value was >0.1 µM), or a positive response was reported without an EC_{50} value. The EC_{50} is the effective concentration that causes half-maximal activation of the receptor.

723 [3] ### Indicates that the substance was uniformly positive in multiple assays; ## indicates that the substance was positive in the majority of assays in which it was
724 tested; # indicates that the substance was positive in the single assay in which it was tested; #- indicates the substance was positive in one assay but was also
725 negative in one or more assays; - indicates that the substance was uniformly negative in multiple assays.
726 [4]Substances were assigned into one or more chemical classes using the U.S. National Library of Medicine's Medical Subject Headings (MeSH; Available:
727 http://www.nlm.nih.gov/mesh), an internationally recognized standardized classification scheme.
728 [5] The number of substances indicated in the binding, agonism and antagonism columns may not add up to the number reflected in the distribution substances
729 column because information on these attributes is not available for all substances on the ICCVAM recommended substances list.
730 [6] P = Substance is presumed to be negative.
731 [7]The total number is greater than the total number of proposed reference substances because some substances were assigned to more than one chemical class.
732

Table 8 Revised ICCVAM Minimum List of 53 Reference Substances For *In Vitro* Estrogenic Receptor Binding and Transcriptional Activation Validation Studies – Distribution of Substances by Chemical Class and Available Activity Data

MeSH[4] Chemical Classes	Distribution of Substances[5]	Binding[1]					Agonism[2]					Antagonism[3]				
		+++	++	+	+-	-	+++	++	+	+-	-	###	##	#	#-	-
Amides	1				1											
Amines	1		1													
Azides	1					P[6]					P					P
Carboxylic Acids	5		1	1	1	1		1	1							1
Esters	1				1				1							1
Flavonoids	8	2	3	1		1	1	1	3	2		2		1	1	3
Heterocyclic Compounds	15	2	4	2	1	1	1	1	3	2	3	2		1	1	4
Hydrocarbons (Cyclic)	5	3	1				1	1	1	1	1	2		1		2
Hydrocarbons (Halogenated)	5		2	1	1				5					1		3
Phenols	8		4	2				3	2					1		1
Phthalic Acids	3				2	1		1	1	1						2
Polycyclic Compounds	3					2					2		1			1
Pyrimindines	2					P					1					P
Salts (Inorganic)	1					P					P					P
Steroids	13	5	2	2	1	2	4	3		3	2					4
Totals[7]	**76**	**10**	**18**	**9**	**8**	**12**	**6**	**11**	**17**	**9**	**12**	**6**	**1**	**5**	**2**	**26**

[1] +++ Indicates that the substance was strongly active as measured by relative binding affinity (RBA) compared to estradiol (RBA value was >1); ++ indicates that the substance was moderately active (RBA value was between 1 and 0.01); + indicates that the substance was weakly active (RBA value was < than 0.01); - indicates that an IC_{50} value was not obtained and thus an RBA value could not be determined; ± indicates an equivocal response (i.e., in different studies, the substance was reported as positive and negative). The inhibitory concentration 50 (IC_{50}) is the concentration of test substances that displaces 50% of the radiolabeled reference estrogen or androgen from the receptor.

[2] +++ Indicates that the substance was strongly active (EC_{50} value was <0.001 μM); ++ indicates that the substance was moderately active (EC_{50} value was between 0.001 and 0.1 μM); + indicates that the substance was weakly active (EC_{50} value was >0.1 μM), or a positive response was reported without an EC_{50} value. The EC_{50} is the effective concentration that causes half-maximal activation of the receptor.

[3] ### Indicates that the substance was uniformly positive in multiple assays; ## indicates that the substance was positive in the majority of assays in which it was tested; # indicates that the substance was positive in the single assay in which it was tested; #- indicates the substance was positive in one assay but was also negative in one or more assays; - indicates that the substance was uniformly negative in multiple assays.

[4] Substances were assigned into one or more chemical classes using the U.S. National Library of Medicine's Medical Subject Headings (MeSH; Available: http://www.nlm.nih.gov/mesh), an internationally recognized standardized classification scheme.

[5] The number of substances indicated in the binding, agonism and antagonism columns may not add up to the number reflected in the distribution substances column because information on these attributes is not available for all substances on the ICCVAM recommended substances list.

[6] P = Substance is presumed to be negative.

[7] The total number is greater than the total number of proposed reference substances because some substances were assigned to more than one chemical class.

Table 9 Revised ICCVAM List of 78 Reference Substances For *In Vitro* Androgenic Receptor Binding and Transcriptional Activation Validation Studies – Distribution of Substances by Chemical Class and Available Activity Data

MeSH[4] Chemical Classes	Distribution of Substances[5]	Binding[1]					Agonism[2]					Antagonism[3]				
		+++	++	+	+-	-	+++	++	+	+-	-	###	##	#	#-	-
Amides	3	1	2						2		1		2	1		
Amines	2					P[6]					P					P
Amino Acids	1					P					P					P
Azides	1					P					P					P
Carboxylic Acids	5					P					1					1
Esters	1					P					1					
Flavonoids	9										2			1		
Heterocyclic Compounds	23	1		1						2	5	1	1	1		2
Hydrocarbons (Cyclic)	6		1								2				2	
Hydrocarbons (Halogenated)	5		2	2				1			3			2	2	
Indoles	1					P					P					P
Imidazoles	1	1								1			1			
Ketones	1					P					P					P
Lactones	1	1							1				1			
Onium Compounds	1					P					P					P
Phenols	8									1	4	3		1	1	
Phthalic Acids	3					P					3					1
Polycyclic Compounds	4			1					1		1			2		
Pyrimindines	3					P					1					P
Salts (Inorganic)	1					P					P					P
Steroids	22	12	4	1		1	3	4	6		4	1	2		1	3
Ureas	1			1					1					1		
Totals[7]	**103**	**16**	**9**	**6**	**0**	**13**	**3**	**5**	**11**	**4**	**36**	**5**	**7**	**9**	**6**	**16**

[1] +++ Indicates that the substance was strongly active as measured by relative binding affinity (RBA) compared to a reference androgen (RBA value was >1); ++ indicates that the substance was moderately active (RBA value was between 1 and 0.01); + indicates that the substance was weakly active (RBA value was < than 0.01); - indicates that an IC_{50} value was not obtained and thus an RBA value could not be determined; ± indicates an equivocal response (i.e., in different studies, the substance was reported as positive and negative). The inhibitory concentration 50 (IC_{50}) is the concentration of test substances that displaces 50% of the radiolabeled reference androgen from the receptor.

[2] +++ Indicates that the substance was strongly active (EC_{50} value was <0.001 µM); ++ indicates that the substance was moderately active (EC_{50} value was between 0.001 and 0.1 µM); + indicates that the substance was weakly active (EC_{50} value was >0.1 µM), or a positive response was reported without an EC_{50} value. The EC_{50} is the effective concentration that causes half-maximal activation of the receptor.

762 [3] ### Indicates that the substance was uniformly positive in multiple assays; ## indicates that the substance was positive in the majority of assays in which it was
763 tested; # indicates that the substance was positive in the single assay in which it was tested; #- indicates the substance was positive in one assay but was also
764 negative in one or more assays; - indicates that the substance was uniformly negative in multiple assays.
765 [4]Substances were assigned into one or more chemical classes using the U.S. National Library of Medicine's Medical Subject Headings (MeSH; Available:
766 http://www.nlm.nih.gov/mesh), an internationally recognized standardized classification scheme.
767 [5] The number of substances indicated in the binding, agonism and antagonism columns may not add up to the number reflected in the distribution substances
768 column because information on these attributes is not available for all substances on the ICCVAM recommended substances list.
769 [6] P = Substance is presumed to be negative.
770 [7]The total number is greater than the total number of proposed reference substances because some substances were assigned to more than one chemical class.
771

Table 10 Revised ICCVAM Minimum List of 44 Reference Substances For *In Vitro* Androgenic Receptor Binding and Transcriptional Activation Validation Studies – Distribution of Substances by Chemical Class and Available Activity Data

MeSH[4] Chemical Classes	Distribution of Substances[5]	Binding[1]					Agonism[2]					Antagonism[3]				
		+++	++	+	+-	-	+++	++	+	+-	-	###	##	#	#-	-
Amides	2	1	1						1				2			
Azides	1					P[6]					P					P
Carboxylic Acids	1					P					P					P
Esters	1										1					
Flavonoids	1					P					P					P
Heterocyclic Compounds	8	1		1						2	3	1	1			2
Hydrocarbons (Cyclic)	2		1								1			1		
Hydrocarbons (Halogenated)	5		2	2				1			3			2	2	
Imidazoles	1	1								1			1			
Lactones	1	1							1				1			
Phenols	3									1	2	1			1	
Phthalic Acids	2										2					
Polycyclic Compounds	3			1							1			2		
Pyrimindines	1										1					
Salts (Inorganic)	1					P					P					P
Steroids	18	11	4	1		1	3	4	6	0	3	1	2		1	3
Ureas	1			1					1					1		
Totals[7]	**52**	**15**	**8**	**6**	**0**	**5**	**3**	**5**	**9**	**4**	**21**	**3**	**7**	**6**	**4**	**9**

[1] +++ Indicates that the substance was strongly active as measured by relative binding affinity (RBA) compared to a reference androgen (RBA value was >1); ++ indicates that the substance was moderately active (RBA value was between 1 and 0.01); + indicates that the substance was weakly active (RBA value was < than 0.01); - indicates that an IC_{50} value was not obtained and thus an RBA value could not be determined; ± indicates an equivocal response (i.e., in different studies, the substance was reported as positive and negative). The inhibitory concentration 50 (IC_{50}) is the concentration of test substances that displaces 50% of the radiolabeled reference estrogen or androgen from the receptor.

[2] +++ Indicates that the substance was strongly active (EC_{50} value was <0.001 μM); ++ indicates that the substance was moderately active (EC_{50} value was between 0.001 and 0.1 μM); + indicates that the substance was weakly active (EC_{50} value was >0.1 μM), or a positive response was reported without an EC_{50} value. The EC_{50} is the effective concentration that causes half-maximal activation of the receptor.

[3] ### Indicates that the substance was uniformly positive in multiple assays; ## indicates that the substance was positive in the majority of assays in which it was tested; # indicates that the substance was positive in the single assay in which it was tested; #- indicates the substance was positive in one assay but was also negative in one or more assays; - indicates that the substance was uniformly negative in multiple assays.

[4] Substances were assigned into one or more chemical classes the U.S. National Library of Medicine's Medical Subject Headings (MeSH; Available: http://www.nlm.nih.gov/mesh), an internationally recognized standardized classification scheme.

[5] The number of substances indicated in the binding, agonism and antagonism columns may not add up to the number reflected in the distribution substances column because information on these attributes is not available for all substances on the ICCVAM recommended substances list.

[6] P = Substance is presumed to be negative.

[7]The total number is greater than the total number of proposed reference substances because some substances were assigned to more than one chemical class.

because it is the active metabolite of tamoxifen and is therefore active in all cell based systems, it is well represented in the scientific literature, and it is listed as a minimum substance to be tested for both ER and AR validation studies.

Replacement substances have been identified that have similar ER or AR binding or agonist TA activity profiles, or that are similarly concordant for antagonist TA activity across studies, and that are readily commercially available and are less expensive. Consideration for replacement substances was also based on whether the substance was on the original list of 122 ICCVAM ED candidate substances classified as "Substances Considered but not Included for Validation" or if the substance is being evaluated for ED activity by the EPA.

Replacement substances have been identified based on the above criteria. Replacements are recommended as follows:

- 4-OH androstenedione for anastrozole
- chrysin for CGS 18320B
- dicofol for fadrozole
- raloxifene for ICI 182,780
- 19-nortestosterone for methyltrienolone
- resveratrol for zearalenone

These proposed replacements have similar ER or AR binding or agonist TA activity profiles, or are similarly concordant for antagonist TA activity across studies. Four substances, 4-OH androstenedione, dicofol, raloxifene, and 19-nortestosterone are on the original list of 122 ICCVAM candidate ED substances. Chrysin and dicofol are being evaluated for ED activity by the EPA. The ER data for resveratrol was obtained from a review of the current scientific literature.

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Appendix A

Original ICCVAM Reference Substances for Validation of *In Vitro* Estrogen and Androgen Binding and TA Assays

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Appendix A Original ICCVAM Reference Substances for the Validation of *In Vitro* ER and AR Binding and TA Assays

Substance	CASRN[a]	MeSH[b] Chemical Class	Product Class	List of 78	Included on Minimum Lists		*In Vitro* Data (NICEATM)[a]			EPA	Proposed *In Vivo* Studies[g,h,I,j]	Comments
							Binding[c]	Transcriptional Activation				
					ER Assays	AR Assays		Agonism[d]	Antag[e,f]			
Substances Proposed for Validation												
Actinomycin D	50-76-0	Heterocyclic Compound, Polycyclic Compound	Laboratory Chemical, Pharmaceutical, Veterinary Agent	†[k]	ER	AR	ı					Inhibits protein synthesis; recommended by the Expert Panel
Ammonium perchlorate	7790-98-9	Amine, Onium Compound	Industrial Chemical, Laboratory Chemical, Pharmaceutical	†	-	-				Y[n]	**	Thyroid disruptor; being considered for testing *in vivo* by EPA
Anastrazole	**120511-73-1**	**Nitrile**	**Pharmaceutical**	†	**-**	**-**		**AR-**		**Y**	**IM**	**Aromatase inhibitor; being tested in vivo by EPA**
4-Androstene dione	63-05-8	Steroid	Pharmaceutical	†	ER	AR	ER+/ AR+++	ER- /AR+++		Y		strong AR agonist; being tested *in vitro* by EPA
Apigenin	520-36-5	Heterocyclic Compound	Dye, Natural Product, Pharmaceutical Intermediate	†	ER	-	ER+++	ER+++	ER#-	Y	**	strong ER agonist; being considered for testing *in vivo* by EPA
Apomorphine	58-00-4	Heterocyclic Compound	Pharmaceutical, Veterinary Agent	†	-	-				Y	IM	dopamine D1/D2 receptor agonist; being tested *in vivo* by EPA
Atrazine	1912-24-9	Heterocyclic Compound	Herbicide	†	ER	AR	ER+/AR+	ER-/AR-	ER-/AR-	Y	M-PA; **	Binds weakly to AR and ER; being tested *in vitro* and *in vivo* by EPA

Substance	CASRN[a]	MeSH[b] Chemical Class	Product Class	List of 78	Included on Minimum Lists		*In Vitro* Data (NICEATM)[a]			EPA	Proposed *In Vivo* Studies[g,h,I,j]	Comments
							Binding[c]	Transcriptional Activation				
					ER Assays	AR Assays		Agonism[d]	Antag[e,f]			
Bicalutamide	90357-06-5	Amide	Pharmaceutical	†	-	AR	AR+++	AR+	AR##			AR antagonist; recommended by the Expert Panel
Bisphenol A	80-05-7	Phenol	Chemical Intermediate, Flame Retardant, Fungicide	†	ER	AR	ER++	ER+/AR-	ER-/AR#-	Y	U; F-PA	weak ER agonist; being tested *in vitro* and *in vivo* by EPA and *in vivo* by OECD
Bisphenol B	77-40-7	Phenol	Chemical Intermediate, Flame Retardant, Fungicide	†	ER	-	ER++	ER++/AR-		Y		ER agonist; being tested *in vitro* by EPA
Butylbenzyl phthalate	85-68-7	Carboxylic Acid, Phthalic Acid	Chemical Intermediate, Plasticizer	†	ER	-	ER±	ER++/AR-	ER-/AR-	Y	**	ER agonist; being considered for testing *in vivo* by EPA
2-*sec*-Butylphenol	89-72-5	Phenol	Chemical Intermediate, Pesticide Intermediate, Plasticizer	†	ER	-	ER+			Y		binds weakly to ER; being tested *in vitro* by EPA
CGS 18320B	**112808-99-8**	**Heterocyclic Compound, Imidazole**	**Pharmaceutical**	†	**-**	**-**					**407**	**Aromatase inhibitor; being tested in vivo by OECD**
Clomiphene citrate	50-41-9	Amine, Carboxylic Acid, Heterocyclic Compound	Pharmaceutical	†	ER	-	ER++			Y		Binds to ER; being tested *in vitro* by EPA; recommended by the Expert Panel
Corticosterone	50-22-6	Steroid	Pharmaceutical	†	ER	AR	ER-/AR+	ER-/AR-		Y		binds weakly to AR; being tested *in vitro* by EPA

Substance	CASRN[a]	MeSH[b] Chemical Class	Product Class	List of 78	Included on Minimum Lists		*In Vitro* Data (NICEATM)[a]			EPA	Proposed *In Vivo* Studies[g,h,I,j]	Comments
							Binding[c]	Transcriptional Activation				
					ER Assays	AR Assays		Agonism[d]	Antag[e,f]			
Coumestrol	479-13-0	Heterocyclic Compound	Natural Product	†	ER	-	ER+++	ER++/AR-	ER-	Y	IM	ER agonist; being tested *in vitro* and *in vivo* by EPA
4-Cumylphenol	599-64-4	Phenol	Chemical Intermediate	†	ER	-		ER+/AR-		Y		weak ER agonist; being tested *in vitro* by EPA
Cycloheximide	66-81-9	Heterocyclic Compound	Fungicide, Pharmaceutical, Veterinary Agent	†	-	-						inhibits protein synthesis; recommended by the Expert Panel as a negative control
Cyproterone acetate	427-51-0	Steroid	Pharmaceutical	†	-	AR	AR+++	ER-/AR+	AR##	Y	IM	AR agonist and antagonist; being tested *in vitro* and *in vivo* by EPA
Daidzein	486-66-8	Flavonoid, Heterocyclic Compound	Natural Product	†	ER	-	ER++	ER+	ER-	Y		weak ER agonist; being tested *in vitro* by EPA
p,p'-DDE*	72-55-9	Hydrocarbon (Halogenated)	Pesticide Intermediate	†	ER	AR	ER±/ AR++	ER+/AR±	ER- /AR#-	Y	H/407; M-PA; IM	weak AR agonist and antagonist; being tested *in vitro* and *in vivo* by EPA and *in vivo* by OECD
o,p'-DDT*	789-02-6	Hydrocarbon (Halogenated)	Pesticide	†	ER	AR	ER++/ AR+	ER+/AR-	ER#/ AR#		U	weak ER agonist and antagonist; weak AR antagonist; being tested *in vivo* by OECD
Dexamethasone	50-02-2	Steroid	Pharmaceutical, Veterinary Agent	†	ER	AR	ER-/AR-	ER±/AR+		Y		weak ER and AR agonist; being tested *in vitro* by EPA

Substance	CASRN[a]	MeSH[b] Chemical Class	Product Class	List of 78	Included on Minimum Lists		*In Vitro* Data (NICEATM)[a]			EPA	Proposed *In Vivo* Studies[g,h,I,j]	Comments
							Binding[c]	Transcriptional Activation				
					ER Assays	AR Assays		Agonism[d]	Antag[e,f]			
Dibenzo[*a,h*] anthracene	53-70-3	Polycyclic Compound	Laboratory Chemical, Natural Product	†	ER	-	ER-	ER-/AR+	ER##			ER antagonist; included as it belongs to an under-represented class of substances
Di-*n*-butyl phthalate	84-74-2	Phthalic Acid	Pesticide Intermediate, Plasticizer	†	ER	AR	ER±	ER+/AR-	ER-	Y	U; M-PA; 1G	ER agonist; being tested *in vivo* by EPA and OECD
Diethylhexyl phthalate	117-81-7	Ester, Phthalic Acid	Cosmetic Ingredient, Industrial Chemical, Plasticizer	†	ER	AR	ER-	AR-		Y		being tested *in vitro* by EPA
Diethylstilbestrol	56-53-1	Hydrocarbon (Cyclic)	Pharmaceutical, Veterinary Agent	†	ER	AR	ER+++/ AR++	ER+++/ AR-	AR#	Y	**	ER agonist; being considered for testing *in vivo* by EPA
5α-Dihydro testosterone***	521-18-6	Steroid	Pharmaceutical	†	ER	AR	ER++/ AR+++	ER+/ AR+++		Y	H	weak ER agonist; strong AR agonist; being tested *in vitro* by EPA and *in vivo* by OECD
17α-Estradiol	57-91-0	Steroid	Pharmaceutical, Veterinary Agent	†	ER	-	ER+++	ER++/AR-		Y		ER agonist; being tested *in vitro* by EPA
17β-Estradiol***	50-28-2	Steroid	Pharmaceutical, Veterinary Agent	†	ER	AR	ER+++/ AR++	ER+++/ AR++	AR##	Y	IM; **; FRS	strong ER agonist; AR agonist and antagonist; being tested *in vitro* and *in vivo* by EPA
Estrone	53-16-7	Steroid	Pharmaceutical, Veterinary Agent	†	ER	AR	ER+++/ AR++	ER+++/ AR++		Y		strong ER agonist; AR agonist; being tested *in vitro* by EPA

Substance	CASRN[a]	MeSH[b] Chemical Class	Product Class	List of 78	Included on Minimum Lists		*In Vitro* Data (NICEATM)[a]			EPA	Proposed *In Vivo* Studies[g,h,I,j]	Comments
					ER Assays	AR Assays	Binding[c]	Transcriptional Activation				
								Agonism[d]	Antag[e,f]			
17α-Ethinyl estradiol	57-63-6	Steroid	Pharmaceutical, Veterinary Agent	†	ER	AR	ER+++/ AR++	ER+++/ AR-		Y	U/407; F-PA	strong ER agonist; being tested *in vitro* and *in vivo* by EPA and *in vivo* by OECD
Ethyl paraben	120-47-8	Carboxylic Acid, Phenol	Pharmaceutical, Preservative	†	ER	-				Y		being tested *in vitro* by EPA
Fadrozole	**102676-47-1**	**Imidazole, Nitrile**	**Pharmaceutical**	†	**ER**	**AR**				**Y**	**F-PA; IM; FRS**	**Aromatase inhibitor; being tested in vivo by EPA**
Fenarimol	60168-88-9	Heterocyclic Compound, Pyrimidine	Fungicide	†	-	-		ER+	ER#	Y	F-PA	Aromatase inhibitor; being tested *in vivo* by EPA
Finasteride	98319-26-7	Steroid	Pharmaceutical	†	-	AR		AR-	AR-	Y	H; M-PA; IM	5α-reductase inhibitor; being tested *in vivo* by EPA and by OECD
Flavone	525-82-6	Flavonoid, Heterocyclic Compound	Natural Product, Pharmaceutical	†	ER	-	ER-	ER±	ER###	Y	M-PA; IM	ER antagonist; being tested *in vivo* by EPA
Fluoranthene	206-44-0	Polycyclic Compound	Industrial Chemical, Laboratory Chemical, Pharmaceutical Intermediate	†	ER	AR	ER-	ER-	ER- /AR#			AR antagonist; included as it belongs to an under-represented class of substances
Fluoxymestrone	76-43-7	Steroid	Pharmaceutical	†	-	AR	AR++	AR+	AR-			weak AR agonist; recommended by the Expert Panel
Flutamide	13311-84-7	Amide	Pharmaceutical, Veterinary Agent	†	-	-	AR++	ER-/AR-	AR#	Y	H/407; M-PA; IM; FRS	AR antagonist; being tested *in vivo* by EPA and by OECD

Substance	CASRN[a]	MeSH[b] Chemical Class	Product Class	List of 78	Included on Minimum Lists		*In Vitro* Data (NICEATM)[a]			EPA	Proposed *In Vivo* Studies[g,h,I,j]	Comments
					ER Assays	AR Assays	Binding[c]	Transcriptional Activation				
								Agonism[d]	Antag[e,f]			
Genistein	446-72-0	Flavonoid, Heterocyclic Compound	Natural Product, Pharmaceutical	†	ER	-	ER++	ER+	ER#		U/407	weak ER agonist and antagonist; being tested *in vivo* by OECD
Haloperidol	52-86-8	Ketone	Pharmaceutical, Veterinary Agent	†	-	-				Y	IM	dopamine D2 receptor antagonist; being tested *in vivo* by EPA
meso-Hexestrol	84-16-2	Steroid	Pharmaceutical, Veterinary Agent	†	ER	-	ER+++	ER+++		Y		strong ER agonist; being tested *in vitro* by EPA
Hydroxy flutamide	52806-53-8	Amide	Pharmaceutical	†	ER	AR	ER±/ AR++	AR+	AR##			AR agonist and antagonist; recommended by the Expert Panel
4-Hydroxy tamoxifen	68047-06-3	Hydrocarbon (Cyclic)	Pharmaceutical	†	ER	AR	ER+++	ER±/AR-	ER###			ER antagonist; recommended by the Expert Panel
ICI 182,780	**129453-61-8**	**Steroid**	**Pharmaceutical**	†	**ER**	**-**	**ER+++**	**ER-/AR-**	**ER###/ AR-**	**Y**	**IM**	**ER antagonist; being tested in vivo by EPA**
Kaempferol	520-18-3	Flavonoid, Heterocyclic Compound	Natural Product	†	ER	-	ER++	ER+	ER-	Y		weak ER agonist; being tested *in vitro* by EPA
Kepone	143-50-0	Hydrocarbon (Halogenated)	Pesticide	†	ER	AR	ER++/ AR++	ER+/AR-	AR#-			binds to ER and AR; included as it belongs to an under-represented class of substances
Ketoconazole	65277-42-1	Heterocyclic Compound	Pharmaceutical	†	-	AR		AR±	AR-	Y	F and M-PA; IM	weak AR agonist; being tested *in vivo* by EPA

Substance	CASRN[a]	MeSH[b] Chemical Class	Product Class	List of 78	Included on Minimum Lists		*In Vitro* Data (NICEATM)[a]			EPA	Proposed *In Vivo* Studies[g,h,I,j]	Comments
					ER Assays	AR Assays	Binding[c]	Transcriptional Activation				
								Agonism[d]	Antag[e,f]			
Linuron	330-55-2	Urea	Herbicide	†	-	AR	AR+	ER-/AR+	AR#	Y	H; M-PA	weak AR agonist and antagonist; being tested *in vitro* and *in vivo* by EPA and *in vivo* by OECD
Medroxy progesterone acetate	71-58-9	Steroid	Pharmaceutical	†	-	AR	AR+++	AR+		Y		weak AR agonist; being tested *in vitro* by EPA
p,p′-Methoxychlor	72-43-5	Hydrocarbon (Halogenated)	Pesticide, Veterinary Agent	†	ER	AR	ER+/AR+	ER+/AR-	ER-/AR#	Y	U; F and M-PA; IM; **; 2G (avian)/FRS	weak ER agonist; AR antagonist; being tested *in vitro* and *in vivo* by EPA and *in vivo* by OECD
Methyl testosterone	58-18-4	Steroid	Pharmaceutical, Veterinary Agent	†	ER	AR	AR+++	ER++/ AR++		Y	H/407; M-PA; **; FRS	ER and AR agonist; being tested *in vivo* by EPA and by OECD
Methyl trienolone* **	**965-93-5**	**Steroid**	**Pharmaceutical**	†	**-**	**AR**	**AR+++**	**ER-/AR+**	**AR-**			**weak AR agonist**
Mifepristone	84371-65-3	Steroid	Pharmaceutical	†	-	AR	AR+++	ER-/AR++	AR###	Y	IM	AR agonist and antagonist; being tested *in vivo* by EPA
Morin	480-16-0	Flavonoid, Heterocyclic Compound	Dye, Natural Product, Pharmaceutical Intermediate	†	ER	-	ER+			Y		binds weakly to ER; being tested *in vitro* by EPA
Nilutamide	63612-50-0	Heterocyclic Compound, Imidazole	Pharmaceutical	†	-	AR	AR+++	AR±	AR##			AR antagonist; recommended by the Expert Panel

Substance	CASRN[a]	MeSH[b] Chemical Class	Product Class	List of 78	Included on Minimum Lists		*In Vitro* Data (NICEATM)[a]			EPA	Proposed *In Vivo* Studies[g,h,I,j]	Comments
							Binding[c]	Transcriptional Activation				
					ER Assays	AR Assays		Agonism[d]	Antag[e,f]			
p-n-Nonylphenol	104-40-5	Phenol	Chemical Intermediate	†	ER	AR	ER++	ER++/AR±	ER#/ AR###	Y	U/407	ER agonist and antagonist; AR antagonist: being tested *in vitro* by EPA and *in vivo* by OECD
Norethynodrel	68-23-5	Steroid	Pharmaceutical	†	ER	-	ER++			Y		Binds to ER; being tested *in vitro* by EPA
4-tert- Octylphenol	140-66-9	Phenol	Chemical Intermediate, Pharmaceutical Intermediate	†	ER	AR	ER++	ER++/AR-		Y		ER agonist; being tested *in vitro* by EPA
Oxazepam	604-75-1	Heterocyclic Compound	Pharmaceutical, Veterinary Agent	†	-	-				Y	IM	enhances thyroid hormone excretion; being tested *in vivo* by EPA
Phenobarbital	57-30-7	Heterocyclic Compound, Pyrimidine	Pharmaceutical, Veterinary Agent	†	ER	AR		ER-/AR-		Y	F and M-PA; IM	enhances thyroid hormone excretion; being tested *in vivo* by EPA
Phenolphthalin	81-90-3	Carboxylic Acid, Phenol	Dye, Laboratory Chemical	†	ER	-	ER+			Y		binds weakly to ER; being tested *in vitro* by EPA
Pimozide	2062-78-4	Heterocyclic Compound	Pharmaceutical	†	-	-				Y	F and M-PA	Dopamine receptor antagonist; being tested *in vivo* by EPA
Procymidone	32809-16-8	Polycyclic Compound	Fungicide	†	-	AR	AR+	ER-/AR-	AR#	Y	H	AR antagonist; being tested *in vitro* by EPA and *in vivo* by OECD
Progesterone	57-83-0	Steroid	Pharmaceutical, Veterinary Agent	†	ER	AR	ER+/ AR+++	ER±/AR+	ER-/AR#-	Y	IM	AR agonist; being tested *in vitro* and *in vivo* by EPA

Substance	CASRN[a]	MeSH[b] Chemical Class	Product Class	List of 78	Included on Minimum Lists		*In Vitro* Data (NICEATM)[a]			EPA	Proposed *In Vivo* Studies[g,h,I,j]	Comments
							Binding[c]	Transcriptional Activation				
					ER Assays	AR Assays		Agonism[d]	Antag[e,f]			
Propylthiouracil	51-52-5	Heterocyclic Compound, Pyrimidine	Pharmaceutical, Veterinary Agent	†	ER	-				Y	407; F and M-PA; **; 2G	inhibits T3/T4 synthesis; being tested *in vivo* by EPA and by OECD
Reserpine	50-55-5	Heterocyclic Compound, Indole	Pharmaceutical, Veterinary Agent	†	-	-				Y	IM	Depletes dopamine; being tested *in vivo* by EPA
Sodium azide	26628-22-8	Azide, Salt (Inorganic)	Chemical Intermediate, Fungicide, Herbicide	†	ER	AR						Cytotoxicant; recommended by the Expert Panel as a negative control
Spironolactone	52-01-7	Lactone, Steroid	Pharmaceutical	†	-	AR	AR+++	AR+	AR##	Y		AR agonist and antagonist; being tested *in vitro* by EPA
Tamoxifen	10540-29-1	Hydrocarbon (Cyclic)	Pharmaceutical	†	ER	-	ER+++	ER±/AR-	ER###			ER antagonist; Tamoxifen citrate is being tested *in vitro* by EPA
Testosterone	58-22-0	Steroid	Pharmaceutical, Veterinary Agent	†	ER	AR	ER±/ AR+++	ER±/ AR+++	AR-	Y	IM	strong AR agonist; being tested *in vitro* and *in vivo* by EPA
12-*O*-Tetradecanoyl phorbol-13-acetate	16561-29-8	Hydrocarbon (Cyclic)	Laboratory Chemical	†	ER	AR						activates ligand independent cell division; recommended by the Expert Panel
L-Thyroxine	51-48-9	Amino Acid	Pharmaceutical, Veterinary Agent	†	-	-					407	thyroid hormone; being tested *in vivo* by OECD
17β-Trenbolone	10161-33-8	Steroid	Pharmaceutical	†	-	AR	AR+++	ER-		Y	H	binds strongly to the AR; being tested *in vivo* by OECD

Substance	CASRN[a]	MeSH[b] Chemical Class	Product Class	List of 78	Included on Minimum Lists		*In Vitro* Data (NICEATM)[a]			EPA	Proposed *In Vivo* Studies[g,h,I,j]	Comments
							Binding[c]	Transcriptional Activation				
					ER Assays	AR Assays		Agonism[d]	Antag[e,f]			
2,4,5-Trichloro phenoxyacetic acid	93-76-5	Carboxylic Acid	Herbicide	†	ER	AR	ER-	ER+		Y		weak ER agonist; being tested *in vitro* by EPA
Vinclozolin	50471-44-8	Heterocyclic Compound	Fungicide	†	ER	AR	ER±/ AR++	ER-/AR-	AR###	Y	H; M-PA; IM; **; 1G/FRS	AR antagonist; being tested *in vitro* and *in vivo* by EPA and *in vivo* by OECD
Zearalenone	**17924-92-4**	**Lactone, Phenol**	**Chemical Intermediate, Veterinary Agent**	†	**ER**	**-**	**ER+++**	**ER++/AR-**	**ER#-**			**ER agonist; included as it belongs to an under-represented class of substances**
Substances Considered but not Included for Validation												
Arochlor 1254	11097-69-1	Hydrocarbon (Halogenated)	Chemical Intermediate, Pesticide Intermediate, Plasticizer		-	-	ER-	ER-		Y	**	does not bind to ER; omitted due to disposal concerns
Bendiocarb	22781-23-3	Carboxylic Acid	Pesticide		-	-		ER-	ER#			ER antagonist but no *in vivo* testing planned
Bisphenol C 2	14868-03-2	Phenol	Chemical Intermediate, Flame Retardant, Fungicide		-	-	ER+++					binds strongly to the ER but no *in vivo* testing planned
4-*tert*-Butylphenol	98-54-4	Phenol	Chemical Intermediate		-	-	ER±	ER+				weak ER agonist but no *in vivo* testing planned
Chlordane	57-74-9	Hydrocarbon (Halogenated)	Pesticide		-	-	ER-	ER+				weak ER agonist but no *in vivo* testing planned

Substance	CASRN[a]	MeSH[b] Chemical Class	Product Class	List of 78	Included on Minimum Lists		*In Vitro* Data (NICEATM)[a]			EPA	Proposed *In Vivo* Studies[g,h,I,j]	Comments
					ER Assays	AR Assays	Binding[c]	Transcriptional Activation				
								Agonism[d]	Antag[e,f]			
4-Chloro-4'-biphenylol	28034-99-3	Hydrocarbon (Cyclic)	-		-	-	ER+	ER+	ER-			weak ER agonist but no *in vivo* testing planned; concern regarding disposal
Chrysin	***480-40-0***	***Flavonoid, Heterocyclic Compound***	***Natural Product***		**-**	**-**				***Y***		***negative for ER and AR binding and no in vivo testing planned***
Cortisol	50-23-7	Steroid	Pharmaceutical, Veterinary Agent		-	-	ER-/AR-	ER-/AR++				AR agonist but no *in vivo* testing planned
Cyanoketone	4248-66-2	Steroid	Pharmaceutical		-	-	AR-					negative for ER and AR binding and no *in vivo* testing planned
p,p'-DDT*	50-29-3	Hydrocarbon (Halogenated)	Pesticide		-	-	ER+/AR+	ER+/AR-	AR##			weak ER agonist; AR antagonist; but no *in vivo* testing planned
Dicofol	***115-32-2***	***Hydrocarbon (Cyclic), Hydrocarbon (Halogenated)***	***Pesticide***		**-**	**-**		***ER+/AR-***	***ER-***	***Y***		***weak ER agonist but no in vivo testing planned***
Droloxifene	82413-20-5	Hydrocarbon (Cyclic)	Pharmaceutical		-	-	ER+++	ER±	ER###			ER antagonist but no *in vivo* testing planned
Equol	531-95-3	Flavonoid, Heterocyclic Compound	Natural Product, Pharmaceutical		-	-	ER++	ER+/AR-				ER agonist but no *in vivo* testing planned
Estriol	50-27-1	Steroid	Pharmaceutical, Veterinary Agent		-	-	ER+++/ AR-	ER++				ER agonist but no *in vivo* testing planned
Fenitrothion	122-14-5	Organo-phosphorus Compound	Pesticide		-	-		AR+	AR##			weak AR agonist but no *in vivo* testing planned

Substance	CASRN[a]	MeSH[b] Chemical Class	Product Class	List of 78	Included on Minimum Lists		*In Vitro* Data (NICEATM)[a]			EPA	Proposed *In Vivo* Studies[g,h,I,j]	Comments
							Binding[c]	Transcriptional Activation				
					ER Assays	AR Assays		Agonism[d]	Antag[e,f]			
Formononetin	485-72-3	Flavonoid, Heterocyclic Compound	Natural Product, Pharmaceutical		-	-	ER±	ER+	ER#			Weak ER agonist; ER antagonist but no *in vivo* testing planned
Genistin	529-59-9	Flavonoid, Heterocyclic Compound	Natural Product		-	-	ER±	ER±	ER-			Weak ER agonist but no *in vivo* testing planned
Heptachlor	76-44-8	Hydrocarbon (Halogenated)	Pesticide, Veterinary Agent		-	-	ER-	ER-				Does not bind to ER and no *in vivo* testing planned
4-Hydroxy androstenedione	***566-48-3***	***Steroid***	***Pharmaceutical***		**-**	**-**	***AR++***			***Y***		***Binds to AR but no in vivo testing planned***
17α-Hydroxy progesterone	68-96-2	Steroid	Pharmaceutical, Veterinary Agent		-	-	AR++	ER-				Binds to AR but no *in vivo* testing planned
Hydroxy toremifene	110503-62-3	Hydrocarbon (Cyclic)	Pharmaceutical		-	-		ER±	ER###			ER antagonist but no *in vivo* testing planned
ICI 164,384	98007-99-9	Steroid	Pharmaceutical		-	-	ER+++	ER±	ER###			ER antagonist but no *in vivo* testing planned
Kaempferide	491-54-3	Flavonoid, Heterocyclic Compound	Natural Product		-	-		ER±	ER###			ER antagonist but no *in vivo* testing planned
Letrozole	112809-51-5	Nitrile	Pharmaceutical		-	-				Y	F-PA (?)	Aromatase inhibitor; questionable whether Letrozole will be tested *in vivo*
Levonorgestrel	797-63-7	Steroid	Pharmaceutical		-	-	AR+++	ER±/ AR+++				Weak ER agonist; strong AR agonist; but no *in vivo* testing planned

Substance	CASRN[a]	MeSH[b] Chemical Class	Product Class	List of 78	Included on Minimum Lists		*In Vitro* Data (NICEATM)[a]			EPA	Proposed *In Vivo* Studies[g,h,I,j]	Comments
					ER Assays	AR Assays	Binding[c]	Transcriptional Activation				
								Agonism[d]	Antag[e,f]			
Lindane	58-89-9	Hydrocarbon (Halogenated)	Pesticide, Veterinary Agent		-	-	ER±	ER+/AR-	AR-			Weak ER agonist but no *in vivo* testing planned
Melengestrol acetate	2919-66-6	Steroid	Pharmaceutical		-	-	AR++	ER+				Weak ER agonist but no *in vivo* testing planned
Mestranol	72-33-3	Steroid	Pharmaceutical		-	-	ER++	ER+				Weak ER agonist but no *in vivo* testing planned
Methyl parathion	298-00-0	Organo-phosphorus Compound	Pesticide		-	-		ER+		Y	2G (avian)	Being tested *in vivo* by EPA, but not considered as it is highly toxic
Mirex	2385-85-5	Hydrocarbon (Halogenated)	Flame Retardant, Pesticide		-	-	ER-	ER-/AR-	AR-			Does not bind to ER or AR and no *in vivo* testing planned
Nafoxidine	1845-11-0	Heterocyclic Compound	Pharmaceutical		-	-	ER++	ER±/AR-				Binds to ER but no *in vivo* testing planned
Naringenin	480-41-1	Flavonoid, Heterocyclic Compound	Natural Product		-	-	ER+	ER+	ER#-			Weak ER agonist but no *in vivo* testing planned
19-Nor testosterone	***434-22-0***	***Steroid***	***Pharmaceutical, Veterinary Agent***		**-**	**-**	***ER++/ AR+++***	***ER±/ AR+++***				***weak ER agonist; AR agonist; but no in vivo testing planned***
4-Octylphenol	1806-26-4	Phenol	Chemical Intermediate, Plasticizer		-	-	ER+	ER+	ER#			Weak ER agonist; ER antagonist but no *in vivo* testing planned
Phloretin	60-82-2	Ketone, Phenol	Natural Product		-	-	ER++	ER+	ER#-			weak ER agonist but no *in vivo* testing planned

Substance	CASRN[a]	MeSH[b] Chemical Class	Product Class	List of 78	Included on Minimum Lists		*In Vitro* Data (NICEATM)[a]			EPA	Proposed *In Vivo* Studies[g,h,I,j]	Comments
					ER Assays	AR Assays	Binding[c]	Transcriptional Activation				
								Agonism[d]	Antag[e,f]			
Pregnenolone	145-13-1	Steroid	Pharmaceutical		-	-	AR±					binds weakly to AR but no *in vivo* testing planned
Raloxifene[m]	***84449-90-1***	***Hydrocarbon (Cyclic)***	***Pharmaceutical***		**-**	**-**	***ER+++***	***ER-***	***ER###***	***Y***		***ER antagonist but no in vivo testing planned***
Resveratrol	***501-36-0***	***Hydrocarbon (Cyclic)***	***Natural Product***		**-**	**-**	***ER+***	***ER++***	***ER#/ AR#***	***N***		***Selected as a replacement substances based on review of the scientific literature***
Simazine	122-34-9	Heterocyclic Compound	Herbicide		-	-	ER-	ER±	ER-			weak ER agonist and no *in vivo* testing planned
β-Sitosterol	5779-62-4	Steroid	Natural Product, Pharmaceutical		-	-	ER-	ER±/AR-				weak ER agonist and no *in vivo* testing planned
Tamoxifen citrate	54965-24-1	Hydrocarbon (Cyclic)	Pharmaceutical		-	-	ER+++	ER-	ER#	Y		ER antagonist; being tested *in vitro* by EPA but no *in vivo* testing planned
Testosterone propionate	57-85-2	Steroid	Pharmaceutical		-	-					H	no binding or TA data; being tested by OECD *in vivo*

Substance	CASRN[a]	MeSH[b] Chemical Class	Product Class	List of 78	Included on Minimum Lists		*In Vitro* Data (NICEATM)[a]			EPA	Proposed *In Vivo* Studies[g,h,I,j]	Comments
					ER Assays	AR Assays	Binding[c]	Transcriptional Activation				
								Agonism[d]	Antag[e,f]			
2,3,7,8, -Tetrachloro dibenzo-*p*-dioxin	1746-01-6	Dioxin, Heterocyclic Compound	Laboratory Chemical		-	-	ER-	ER++/AR-	ER###/ AR#	Y	**	ER agonist and antagonist; not considered due to extreme toxicity
2',4',6',-Trichloro-4-biphenylyol	14962-28-8	Hydrocarbon (Cyclic), Hydrocarbon (Halogenated)	Laboratory Chemical		-	-	ER+++	ER+	ER#-			weak ER agonist; no *in vivo* testing planned; concern regarding disposal
α-Zearalanol	26538-44-3	Lactone, Phenol	Natural Product		-	-	ER+++	ER++/AR-	ER##			ER agonist and antagonist; no *in vivo* testing planned
β-Zearalanol	71030-11-0	Lactone, Phenol	Natural Product		-	-	ER+++	ER+				ER agonist; no *in vivo* testing planned

Substances listed in bolded text were originally included on the ICCVAM List of Reference Substances ER/AR Binding and TA Activation Assays, but have been replaced because of lack of commercial availability or excessive cost.
Substances that are both bolded and italicized are replacements for the substances that were not commercially available or had excessive cost.
EPA = United States Environmental Protection Agency
OECD = Organisation for Economic Co-operation and Development
**p,p*'-DDE =1,1-Dichloro-2,2-di(*p*-chlorophenyl)ethylene; *o,p*'-DDT =1,1,1-Trichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)ethane; *p,p*'-DDT =1,1,1-Trichloro-2,2-di(4-chlorophenyl)ethane
** Indicates that a particular substance has been recommended for testing in the *in vivo* assay, *in utero* through lactation.
*** 17β-Estradiol is the recommended positive control substance for the ER binding and ER TA assays; for AR binding, 5α Dihydrotestosterone is the recommended positive control if a purified AR protein is used, while Methyltrienolone or Mibolerone is recommended if intact cells, or cytosol is used. For AR TA assays, either 5α Dihydrotestosterone or Methyltrienolone is recommended as the positive control.
[a] CASRN = Chemical Abstracts Service Registry Number
[b]MeSH = Medical Subject Headings, information on chemical class criteria can be obtained at www.nlm.nih.gov/MeSH
[c] +++ Indicates that the substance was strongly active as measured by the relative binding affinity (RBA) (RBA value was >1); ++ indicates that the substance was moderately active (RBA value was between 1 and 0.01); + indicates that the substance was weakly active (RBA value was < than 0.01); - indicates that an IC_{50} value was not obtained and thus an RBA value could not be determined; ± indicates an equivocal response (i.e., in different studies, the substance was reported as positive and negative). The inhibitory concentration 50 (IC_{50}) is the concentration of test substances that displaces 50% of the radiolabeled reference estrogen or androgen from the receptor.

[d] +++ Indicates that the substance was strongly active (EC_{50} value was <0.001 □M); ++ indicates that the substance was moderately active (EC_{50} value was between 0.001 and 0.1 □M); + indicates that the substance was weakly active (EC_{50} value was >0.1 □M), or a positive response was reported without an EC_{50} value. The EC_{50} is the effective concentration that causes half-maximal activation of the receptor.
[e] Antag = Antagonist
[f] ### Indicates that the substance was uniformly positive in multiple assays; ## indicates that the substance was positive in the majority of assays in which it was tested; # indicates that the substance was positive in the single assay in which it was tested; #- indicates the substance was positive in one assay but was also negative in one or more assays; - indicates that the substance was uniformly negative in multiple assays.
[g] In the Uterotrophic Assay, the weight of the uterus is determined after exposure of the rat or mouse to the test substance for three days. In the Hershberger assay, sex accessory gland weights are determined in castrated male rats 4-7 days after treatment of the animals with the test substance (agonistic response) or the test substance with testosterone (antagonistic response). The 407 protocol assesses the effect on all organs including the reproductive organs, of varying concentrations of the test substance administered daily to seven week-old female rats for 28 days. After treatment, the estrus cycle is evaluated by daily vaginal smears for 5 days while treatment is continued.
[h] In the Intact male assay (IM), adult male rats (70-90 days of age) are dosed daily by intraperitoneal injection for 14 days and sacrificed 24 hours after the last dose. The testes, epididymes, seminal vesicles, and prostate are weighed. One cauda epididymis is weighed and the sperm found in this cauda are evaluated for motility and concentration. One testis, epididymis, and thyroid gland are fixed for histological evaluation. Blood hormone levels are measured. This assay detects effects on male reproductive organs that are sensitive to antiandrogens and substances that interfere with testosterone biosynthesis. The male pubertal assay (M-PA) measures the age of preputial separation (PPS). Androgens accelerate and antiandrogens and estrogens delay PPS. Animals are dosed daily by gavage beginning one week before puberty (40 days of age). The rats are sacrificed and all the reproductive tissues are weighed. Histopathological analysis of the thyroid is performed and blood levels of the thyroid hormone are measured. In the female (F), the Pubertal Assay (F-PA) measures the time it takes for the vaginal opening to be observed following single or multiple daily treatments from 21 days of age (weaning).
[i] The *in utero* through lactation assay (Assesses post-natal development of neonates after *in utero* and lactational exposure) has been recommended, but the EPA has not made a decision for its further development or for validation.
[j] FRS = Fish Reproductive Screen; 1G = one generation; 2G = two generation.
[k] † Indicates that a substance was included in the ICCVAM list of 78 Reference Substances for the Validation of *In Vitro* and *In Vivo* ER and AR Binding and TA Assays.
[l] Empty cells indicate that no relevant data were identified and no validation tests are planned for that substance in that particular assay.
[m] Raloxifene may act as an agonist in some *in vitro* systems.
[n] Y indicates that this substance is being evaluated for ED activity by the EPA.

Appendix B

Revised ICCVAM List of Reference Substances for Validation of *In Vitro* Binding and TA Assays

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Appendix B-1

Revised ICCVAM List of Reference Substances for Validation of *In Vitro* ER Binding Assays (Sorted by ER Binding Activity and Substance Name)

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Appendix B-1 Revised ICCVAM List of Reference Substances for Validation of *In Vitro* ER Binding Assays (Sorted by ER Binding Activity and Substance Name)

Substance	CASRN[a]	MW[b]	EPA Reference Chemicals	ER Binding Activity[c]	Solubility in Water[d]	Solubility in DMSO[d]	log Kow[d]	Total Cost Per 500mg[c,f]	MESH Chemical Class[g]	Product Class
Apigenin	520-36-5	270.2	N[j]	+++	183 mg/L @ 25° C	27 mg/ml @ 25° C	-[h]	$790	Heterocyclic Compound	Dye, Natural Product, Pharmaceutical Intermediate
Coumestrol	479-13-0	268.2	Y[k]	+++	practically insoluble	-	-	$1,550	Heterocyclic Compound	Natural Product
Diethylstilbestrol	56-53-1	268.4	Y	+++	12 mg/L @ 25° C	-	5.07	$47	Hydrocarbon (Cyclic)	Pharmaceutical, Veterinary Agent
17a-Estradiol	57-91-0	272.4	Y	+++	3.9 mg/L	-	3.94	$230	Steroid	Pharmaceutical, Veterinary Agent
17a-Ethinyl estradiol	57-63-6	296.4	Y	+++	insoluble	-	3.67	$35	Steroid	Pharmaceutical, Veterinary Agent
17ß-Estradiol	50-28-2	272.4	Y	+++	3.60 mg/L @ 27 °C	soluble	4.01	$151	Steroid	Pharmaceutical, Veterinary Agent
Estrone	53-16-7	270.4	Y	+++	0.003 g/ 100 mL @ 25° C	-	3.13	$14	Steroid	Pharmaceutical, Veterinary Agent
meso-Hexestrol	84-16-2	270.4	Y	+++	-	-	-	$35	Steroid	Pharmaceutical, Veterinary Agent
4-Hydroxytamoxifen	68047-06-3	387.5	N	+++	practically insoluble	soluble	-	$6,090	Hydrocarbon (Cyclic)	Pharmaceutical
Raloxifene[i]	82640-04-8	510.1	N	+++	insoluble	28 mg/ml	-	$235	Hydrocarbon (Cyclic)	Pharmaceutical
Tamoxifen	10540-29-1	371.5	Y	+++	practically insoluble	soluble	-	$125	Hydrocarbon (Cyclic)	Pharmaceutical

Substance	CASRN[a]	MW[b]	EPA Reference Chemicals	ER Binding Activity[c]	Solubility in Water[d]	Solubility in DMSO[d]	log Kow[d]	Total Cost Per 500mg[e,f]	MESH Chemical Class[g]	Product Class
Bisphenol A	**80-05-7**	**228.3**	**Y**	**++**	**120 mg/L @ 25° C**	**-**	**3.32**	**$12**	**Phenol**	**Chemical Intermediate, Flame Retardant, Fungicide**
Bisphenol B	**77-40-7**	**242.3**	**Y**	**++**	**1 g in 50 mL**	**-**	**2.30**	**$110**	**Phenol**	**Chemical Intermediate, Flame Retardant, Fungicide**
Clomiphene citrate	**50-41-9**	**598.1**	**Y**	**++**	**slightly soluble**	**-**	**-**	**$45**	**Amine, Carboxylic Acid, Heterocyclic Compound**	**Pharmaceutical**
Daidzein	**486-66-8**	**254.2**	**Y**	**++**	**practically insoluble**	**10 mg/ml**	**-**	**$735**	**Flavonoid, Heterocyclic Compound**	**Natural Product**
o,p'* –DDT	**789-02-6**	**354.5**	**Y**	**++**	**0.085 mg/L @ 25° C**	**-**	**6.79**	**$714**	**Hydrocarbon (Halogenated)**	**Pesticide**
5a-Dihydrotestosterone	**521-18-6**	**290.4**	**Y**	**++**	**practically insoluble**	**-**	**3.55**	**$27**	**Steroid**	**Pharmaceutical**
Genistein	**446-72-0**	**270.2**	**Y**	**++**	**insoluble**	**50 mg/ml @ 25° C**	**2.84**	**$943**	**Flavonoid, Heterocyclic Compound**	**Natural Product, Pharmaceutical**
Kaempferol	**520-18-3**	**286.2**	**Y**	**++**	**slightly soluble**	**-**	**1.96**	**$390**	**Flavonoid, Heterocyclic Compound**	**Natural Product**
Kepone	**143-50-0**	**490.6**	**Y**	**++**	**2.70 mg/L @ 25 °C**	**-**	**5.41**	**$123**	**Hydrocarbon (Halogenated)**	**Pesticide**
***p* -n -Nonylphenol**	**104-40-5**	**220.4**	**Y**	**++**	**-**	**-**	**5.76**	**$192**	**Phenol**	**Chemical Intermediate**
Norethynodrel	**68-23-5**	**298.4**	**Y**	**++**	**practically insoluble**	**-**	**3.51**	**$78**	**Steroid**	**Pharmaceutical**
19-Nortestosterone	434-22-0	274.4	N	++	-	-	-	$90	Steroid	Pharmaceutical, Veterinary Agent

Substance	CASRN[a]	MW[b]	EPA Reference Chemicals	ER Binding Activity[c]	Solubility in Water[d]	Solubility in DMSO[d]	log Kow[d]	Total Cost Per 500mg[e,f]	MESH Chemical Class[g]	Product Class
4-*tert*-Octylphenol	140-66-9	206.3	Y	++	-	-	-	$28	Phenol	Chemical Intermediate, Pharmaceutical Intermediate
p,p' –DDE*	72-55-9	318.0	Y	+-	0.04 mg/L 25 °C	-	6.51	$94	Hydrocarbon (Halogenated)	Pesticide Intermediate
Hydroxyflutamide	52806-53-8	292.2	N	+-	27.5 mg/L @ 25 °C	-	2.7	$2,941	Amide	Pharmaceutical
Butylbenzyl phthalate	85-68-7	312.4	Y	+-	2.69 mg/L @ 25° C	-	4.91	$79	Carboxylic Acid, Phthalic Acid	Chemical Intermediate, Plasticizer
Di - *n* -butyl phthalate	84-74-2	278.3	Y	+-	-	-	-	$34	Ester, Phthalic Acid	Cosmetic Ingredient, Industrial Chemical, Plasticizer
Testosterone	58-22-0	288.4	N	+-	practically insoluble	soluble	-	$26	Steroid	Pharmaceutical, Veterinary Agent
Vinclozolin	50471-44-8	286.1	Y	+-	1000 mg/L @ 20 °C	-	3.10	$41	Heterocyclic Compound	Fungicide
4-Androstenedione	63-05-8	286.4	N	+	57.8 mg/mL 25 °C	-	2.75	$53	Steroid	Pharmaceutical
Atrazine	1912-24-9	215.7	Y	+	34.7 mg/L @ 26° C	183 g/kg @ 25° C	2.61	$68	Heterocyclic Compound	Herbicide
2-*sec*-Butylphenol	89-72-5	150.2	N	+	insoluble	-	3.27	$16	Phenol	Chemical Intermediate, Pesticide Intermediate, Plasticizer
p,p'- Methoxychlor	72-43-5	345.7	Y	+	30.5 mg/L @ 25 °C	-	5.08	$17	Hydrocarbon (Halogenated)	Pesticide, Veterinary Agent
Morin	480-16-0	302.2	Y	+	250 mg/L @ 25 °C	-	1.54	$14	Flavonoid, Heterocyclic Compound	Dye, Natural Product, Pharmaceutical Intermediate

Substance	CASRN[a]	MW[b]	EPA Reference Chemicals	ER Binding Activity[c]	Solubility in Water[d]	Solubility in DMSO[d]	log Kow[d]	Total Cost Per 500mg[e,f]	MESH Chemical Class[g]	Product Class
Phenolphthalin	81-90-3	320.3	Y	+	175 mg/L @ 20 °C	-	3.95	$26	Carboxylic Acid, Phenol	Dye, Laboratory Chemical
Progesterone	57-83-0	314.5	Y	+	8.8 mg/L @ 25 °C	-	3.87	$25	Steroid	Pharmaceutical, Veterinary Agent
Resveratrol	501-36-0	228.2	N	+	-	-	3.08	$226	Hydrocarbon (Cyclic)	Natural Product
Corticosterone	50-22-6	346.5	Y	-	199 mg/L @ 25° C	-	-	$47	Steroid	Pharmaceutical
Dexamethasone	50-02-2	392.5	Y	-	10 mg/100mL @ 25 °C	-	-	$352	Steroid	Pharmaceutical, Veterinary Agent
Dibenzo[*a,h*]anthracene	53-70-3	278.4	Y	-	practically insoluble	-	6.5	$352	Polycyclic Compound	Laboratory Chemical, Natural Product
Flavone	525-82-6	222.2	N	-	0.1 mg/L @ 25° C	-	5.08	$48	Flavonoid, Heterocyclic Compound	Natural Product, Pharmaceutical
Fluoranthene	206-44-0	202.3	Y	-	0.2 mg/L	soluble	5.16	$21	Polycyclic Compound	Industrial Chemical, Laboratory Chemical, Pharmaceutical Intermediate
Diethylhexyl phthalate	117-81-7	330.2	Y	-	0.285 mg/L @ 24 °C	-	7.6	$26	Phthalic Acid	Pesticide Intermediate, Plasticizer
2,4,5-Trichlorophenoxyacetic acid	93-76-5	255.5	Y	-	278 mg/L	-	3.31	$48	Carboxylic Acid	Herbicide
Actinomycin D	50-76-0	1255.4	Y	n.d.[l]	1 g/L at 37 °C	10 mg/ml	3.21 @ pH of 7.4	$2,285	Heterocyclic Compound, Polycyclic Compound	Laboratory Chemical, Pharmaceutical, Veterinary Agent

Substance	CASRN[a]	MW[b]	EPA Reference Chemicals	ER Binding Activity[c]	Solubility in Water[d]	Solubility in DMSO[d]	log Kow[d]	Total Cost Per 500mg[e,f]	MESH Chemical Class[g]	Product Class
Ammonium perchlorate	7790-98-9	117.5	N	n.d.	200 g/L at 25 ˚C	-	-	$55	Amine, Onium Compound	Industrial Chemical, Laboratory Chemical, Pharmaceutical
4-Hydroxy androstenedione	566-48-3	302.4	Y	n.d.	-	-	-	$107	Steroid	Pharmaceutical
Apomorphine	58-00-4	267.3	Y	n.d.	1.660 g/mL	-	2.3	$428	Heterocyclic Compound	Pharmaceutical, Veterinary Agent
Bicalutamide	90357-06-5	430.4	Y	n.d.	5 mg/L	-	-	$436	Amide	Pharmaceutical
Chrysin	***480-40-0***	***254.24***	***Y***	***n.d.***	***84 mg/L @ 25 °C***	**-**	***3.52***	***$60***	***Flavonoid, Heterocyclic Compound***	***Natural Product***
4-Cumylphenol	**599-64-4**	**212.3**	**N**	**n.d.**	**insoluble**	**-**	**-**	**$24**	**Phenol**	**Chemical Intermediate**
Cycloheximide	66-81-9	281.4	N	n.d.	0.00021 mg/L @ 25 ˚C	-	0.55	$45	Heterocyclic Compound	Fungicide, Pharmaceutical, Veterinary Agent
Cyproterone acetate	427-51-0	416.9	Y	n.d.	-	-	-	$268	Steroid	Pharmaceutical
Dicofol	*115-32-2*	*370.489*	*Y*	*n.d.*	*1.2 mg/L @24°C*	-	*4.28*	*$88*	*Hydrocarbon (Cyclic), Hydrocarbon (Halogenated)*	*Pesticide*
Fenarimol	60168-88-9	331.2	Y	n.d.	14 mg/L @ 25˚ C	-	3.6	$375	Heterocyclic Compound, Pyrimidine	Fungicide
Finasteride	98319-26-7	372.5	Y	n.d.	-	-	-	$377	Steroid	Pharmaceutical
Fluoxymestrone	76-43-7	336.4	Y	n.d.	practically insoluble	-	2.38	$131	Steroid	Pharmaceutical
Flutamide	13311-84-7	276.2	Y	n.d.	9.45 mg/L @ 25 °C	soluble	3.35	$22	Amide	Pharmaceutical, Veterinary Agent
Haloperidol	52-86-8	375.9	Y	n.d.	1.4 mg/L @ 25 °C	soluble	4.3	$54	Ketone	Pharmaceutical, Veterinary Agent
Ketoconazole	65277-42-1	531.4	Y	n.d.	0.087 mg/L @ 25 °C	-	4.35	$380	Heterocyclic Compound	Pharmaceutical
Linuron	330-55-2	249.1	Y	n.d.	75 mg/L @ 25 °C	-	3.2	$124	Urea	Herbicide

Substance	CASRN[a]	MW[b]	EPA Reference Chemicals	ER Binding Activity[c]	Solubility in Water[d]	Solubility in DMSO[d]	log Kow[d]	Total Cost Per 500mg[e,f]	MESH Chemical Class[g]	Product Class
Medroxyprogesterone acetate	71-58-9	386.5	Y	n.d.	practically insoluble	-	-	$105	Steroid	Pharmaceutical
Mifepristone (Mifeprex)	84371-65-3	429.6	Y	n.d.	insoluble	-	-	$262	Steroid	Pharmaceutical
Nilutamide	63612-50-0	317.2	Y	n.d.	insoluble	-	-	$45	Heterocyclic Compound, Imidazole	Pharmaceutical
Oxazepam	604-75-1	286.7	Y	n.d.	179 mg/L	-	2.24	$463	Heterocyclic Compound	Pharmaceutical, Veterinary Agent
Ethyl paraben	**120-47-8**	**166.2**	**Y**	**n.d.**	**885 mg/L @ 25 °C**	**-**	**2.47**	**$8**	**Carboxylic Acid, Phenol**	**Pharmaceutical, Preservative**
Phenobarbital	**50-06-6**	**232.2**	**Y**	**n.d.**	**1300 mg/L @ 25 °C**	**-**	**1.47**	**$56**	**Heterocyclic Compound, Pyrimidine**	**Pharmaceutical, Veterinary Agent**
Pimozide	2062-78-4	461.6	Y	n.d.	insoluble	18 mg/mL	-	$45	Heterocyclic Compound	Pharmaceutical
Procymidone	32809-16-8	284.1	Y	n.d.	4.5 mg/L @ 25 °C	-	-	$110	Polycyclic Compound	Fungicide
Propylthiouracil	**51-52-5**	**170.2**	**Y**	**n.d.**	**1204 mg/L @ 25 °C**	**-**	**-**	**$28**	**Heterocyclic Compound, Pyrimidine**	**Pharmaceutical, Veterinary Agent**
Reserpine	50-55-5	608.7	Y	n.d.	73 mg/L @ 30 °C	-	-	$76	Heterocyclic Compound, Indole	Pharmaceutical, Veterinary Agent
Sodium azide	**26628-22-8**	**65.0**	**Y**	**n.d.**	**41 g/100 mL @ 15 °C**	**-**	**-**	**$17**	**Azide, Salt (inorganic)**	**Chemical Intermediate, Fungicide, Herbicide**
Spironolactone	52-01-7	416.6	Y	n.d.	22 mg/L @ 25 °C	soluble	2.78	$38	Lactone, Steroid	Pharmaceutical
Methyl testosterone	**58-18-4**	**302.5**	**Y**	**n.d.**	**-**	**-**	**3.32**	**$26**	**Steroid**	**Pharmaceutical, Veterinary Agent**
12-*O*-Tetradecanoylphorbol-13-acetate	**16561-29-8**	**616.8**	**N**	**n.d.**	**-**	**soluble**	**-**	**$11,200**	**Hydrocarbon (Cyclic)**	**Laboratory Chemical**
L-Thyroxine	51-48-9	776.9	Y	n.d.	slightly soluble	-	-	$65	Amino Acid	Pharmaceutical, Veterinary Agent
17b-Trenbolone	10161-33-8	270.4	Y	n.d.	-	-	-	$130	Steroid	Pharmaceutical

Substances listed in bolded text are included on the ICCVAM Minimum List of Substances for Validation of ER binding and TA Assays.
**p,p'*-DDE =1,1-Dichloro-2,2-di(*p*-chlorophenyl)ethylene; *o,p'*-DDT =1,1,1-Trichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)ethane
[a] CASRN = Chemical Abstracts Service Registry Number
[b] MW = Molecular Weight
[c] +++ Indicates that the substance was strongly active as measured by the relative binding affinity (RBA) (RBA value was >1); ++ indicates that the substance was moderately active (RBA value was between 1 and 0.01); + indicates that the substance was weakly active (RBA value was < than 0.01); - indicates that an IC_{50} value was not obtained and thus an RBA value could not be determined; ± indicates an equivocal response (i.e., in different studies, the substance was reported as positive and negative). The inhibitory concentration 50 (IC50) is the concentration of test substances that displaces 50% of the radiolabeled reference estrogen or androgen from the receptor.
[d] Information on Solubility in Water, Solubility in DMSO, and log $K_{ow,}$ were obtained from the National Library of Medicine's ChemIDplus http://chem.sis.nlm.nih.gov/chemidplus/, and from manufacturer Materials Safety Data Sheets (MSDS).
[e] 500 mg is the expected minimum amount of substance required per laboratory to conduct an endocrine disruptor (ED) validation study.
[f] Pricing information was obtained from vendors during October of 2005 and reflects the cost of 500 mg of substance, or the minimum amount sold.
[g] MeSH = Medical Subject Headings, information on chemical class criteria can be obtained at www.nlm.nih.gov/MeSH
[h] A "-" in the fields for Solubility in Water, Solubility in DMSO or low K_{ow} indicates that there is no data for this field.
[i] Raloxifene may act as an agonist in some *in vitro* systems.
[j] N indicates that this substance is not included on the EPA reference chemical list.
[k] Y indicates that this substance is included on the EPA reference chemical list.
[l] n.d. indicates that no relevant data were identified.

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Appendix B-2

Revised ICCVAM List of Reference Substances for Validation of *In Vitro* ER TA Agonist Assays (Sorted by ER Agonist Activity and Substance Name)

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Appendix B-2 Revised ICCVAM List of Reference Substances for Validation of *In Vitro* ER TA Agonist Assays (Sorted by ER TA Agonist Activity and Substance Name)

Substance	CASRN[a]	MW[b]	EPA Reference Chemicals	ER Agonist Activity[c]	Solubility in Water[d]	Solubility in DMSO[d]	log K_{ow}[d]	Total Cost Per 500mg[e,f]	MESH Chemical Class[g]	Product Class
Apigenin	520-36-5	270.2	N[j]	+++	183 mg/L @ 25° C	27 mg/ml @ 25° C	-[h]	$790	Heterocyclic Compound	Dye, Natural Product, Pharmaceutical Intermediate
Diethylstilbestrol	56-53-1	268.4	Y[k]	+++	12 mg/L @ 25° C	-	5.07	$47	Hydrocarbon (Cyclic)	Pharmaceutical, Veterinary Agent
17a-Ethinyl estradiol	57-63-6	296.4	Y	+++	insoluble	-	3.67	$35	Steroid	Pharmaceutical, Veterinary Agent
17ß-Estradiol	50-28-2	272.4	Y	+++	3.60 mg/L @ 27 °C	soluble	4.01	$151	Steroid	Pharmaceutical, Veterinary Agent
Estrone	53-16-7	270.4	Y	+++	0.003 g/ 100 mL @ 25° C	-	3.13	$14	Steroid	Pharmaceutical, Veterinary Agent
meso-Hexestrol	84-16-2	270.4	Y	+++	-	-	-	$35	Steroid	Pharmaceutical, Veterinary Agent
Bisphenol B	77-40-7	242.3	Y	++	1 g in 50 mL	-	2.30	$110	Phenol	Chemical Intermediate, Flame Retardant, Fungicide
Coumestrol	479-13-0	268.2	Y	++	practically insoluble	-	-	$1,550	Heterocyclic Compound	Natural Product
5a-Dihydrotestosterone	521-18-6	290.4	Y	++	practically insoluble	-	3.55	$27	Steroid	Pharmaceutical

Substance	CASRN[a]	MW[b]	EPA Reference Chemicals	ER Agonist Activity[c]	Solubility in Water[d]	Solubility in DMSO[d]	log K_{ow}[d]	Total Cost Per 500mg[e,f]	MESH Chemical Class[g]	Product Class
17a-Estradiol	**57-91-0**	**272.4**	**Y**	**++**	**3.9 mg/L**	**-**	**3.94**	**$230**	**Steroid**	**Pharmaceutical, Veterinary Agent**
***p* -n -Nonylphenol**	**104-40-5**	**220.4**	**Y**	**++**	**-**	**-**	**5.76**	**$192**	**Phenol**	**Chemical Intermediate**
4-*tert*-Octylphenol	**140-66-9**	**206.3**	**Y**	**++**	**-**	**-**	**-**	**$28**	**Phenol**	**Chemical Intermediate, Pharmaceutical Intermediate**
Butylbenzyl phthalate	**85-68-7**	**312.4**	**Y**	**++**	**2.69 mg/L @ 25° C**	**-**	**4.91**	**$79**	**Carboxylic Acid, Phthalic Acid**	**Chemical Intermediate, Plasticizer**
Resveratrol	**501-36-0**	**228.2**	**N**	**++**	**-**	**-**	**3.08**	**$226**	**Hydrocarbon (Cyclic)**	**Natural Product**
Methyl testosterone	**58-18-4**	**302.5**	**Y**	**++**	**-**	**-**	**3.32**	**$26**	**Steroid**	**Pharmaceutical, Veterinary Agent**
Dexamethasone	**50-02-2**	**392.5**	**Y**	**+-**	**10 mg/100mL @ 25 °C**	**-**	**-**	**$352**	**Steroid**	**Pharmaceutical, Veterinary Agent**
Flavone	**525-82-6**	**222.2**	**N**	**+-**	**0.1 mg/L @ 25° C**	**-**	**5.08**	**$48**	**Flavonoid, Heterocyclic Compound**	**Natural Product, Pharmaceutical**
4-Hydroxytamoxifen	**68047-06-3**	**387.5**	**N**	**+-**	**practically insoluble**	**soluble**	**-**	**$6,090**	**Hydrocarbon (Cyclic)**	**Pharmaceutical**
19-Nortestosterone	434-22-0	274.4	N	+-	-	-	-	$90	Steroid	Pharmaceutical, Veterinary Agent
Diethylhexyl phthalate	**117-81-7**	**330.2**	**Y**	**+-**	**0.285 mg/L @ 24 °C**	**-**	**7.6**	**$26**	**Phthalic Acid**	**Pesticide Intermediate, Plasticizer**

Substance	CASRN[a]	MW[b]	EPA Reference Chemicals	ER Agonist Activity[c]	Solubility in Water[d]	Solubility in DMSO[d]	log K_{ow}[d]	Total Cost Per 500mg[e,f]	MESH Chemical Class[g]	Product Class
Progesterone	**57-83-0**	**314.5**	**Y**	**+-**	**8.8 mg/L @ 25 ˚C**	**-**	**3.87**	**$25**	**Steroid**	**Pharmaceutical, Veterinary Agent**
Tamoxifen	**10540-29-1**	**371.5**	**Y**	**+-**	**practically insoluble**	**soluble**	**-**	**$125**	**Hydrocarbon (Cyclic)**	**Pharmaceutical**
Testosterone	**58-22-0**	**288.4**	**N**	**+-**	**practically insoluble**	**soluble**	**-**	**$26**	**Steroid**	**Pharmaceutical, Veterinary Agent**
Bisphenol A	**80-05-7**	**228.3**	**Y**	**+**	**120 mg/L @ 25˚ C**	**-**	**3.32**	**$12**	**Phenol**	**Chemical Intermediate, Flame Retardant, Fungicide**
4-Cumylphenol	**599-64-4**	**212.3**	**N**	**+**	**insoluble**	**-**	**-**	**$24**	**Phenol**	**Chemical Intermediate**
Daidzein	**486-66-8**	**254.2**	**Y**	**+**	**practically insoluble**	**10 mg/ml**	**-**	**$735**	**Flavonoid, Heterocyclic Compound**	**Natural Product**
p,p'*–DDE	**72-55-9**	**318.0**	**Y**	**+**	**0.04 mg/L 25 °C**	**-**	**6.51**	**$94**	**Hydrocarbon (Halogenated)**	**Pesticide Intermediate**
o,p'*–DDT	**789-02-6**	**354.5**	**Y**	**+**	**0.085 mg/L @ 25˚ C**	**-**	**6.79**	**$714**	**Hydrocarbon (Halogenated)**	**Pesticide**
Dicofol	**115-32-2**	**370.489**	**Y**	**+**	**1.2 mg/L @24°C**	**-**	**4.28**	**$88**	**Hydrocarbon (Cyclic), Hydrocarbon (Halogenated)**	**Pesticide**
Fenarimol	60168-88-9	331.2	Y	+	14 mg/L @ 25˚ C	-	3.6	$375	Heterocyclic Compound, Pyrimidine	Fungicide

Substance	CASRN[a]	MW[b]	EPA Reference Chemicals	ER Agonist Activity[c]	Solubility in Water[d]	Solubility in DMSO[d]	log K_{ow}[d]	Total Cost Per 500mg[e,f]	MESH Chemical Class[g]	Product Class
Genistein	446-72-0	270.2	Y	+	insoluble	50 mg/ml @ 25° C	2.84	$943	Flavonoid, Heterocyclic Compound	Natural Product, Pharmaceutical
Kaempferol	520-18-3	286.2	Y	+	slightly soluble	-	1.96	$390	Flavonoid, Heterocyclic Compound	Natural Product
Kepone	143-50-0	490.6	Y	+	2.70 mg/L @ 25 °C	-	5.41	$123	Hydrocarbon (Halogenated)	Pesticide
p,p'- Methoxychlor	72-43-5	345.7	Y	+	30.5 mg/L @ 25 °C	-	5.08	$17	Hydrocarbon (Halogenated)	Pesticide, Veterinary Agent
Di - *n* -butyl phthalate	84-74-2	278.3	Y	+	-	-	-	$34	Ester, Phthalic Acid	Cosmetic Ingredient, Industrial Chemical, Plasticizer
2,4,5-Trichlorophenoxyacetic acid	93-76-5	255.5	Y	+	278 mg/L	-	3.31	$48	Carboxylic Acid	Herbicide
4-Androstenedione	63-05-8	286.4	N	-	57.8 mg/mL 25 °C	-	2.75	$53	Steroid	Pharmaceutical
Atrazine	1912-24-9	215.7	Y	-	34.7 mg/L @ 26° C	183 g/kg @ 25° C	2.61	$68	Heterocyclic Compound	Herbicide
Corticosterone	50-22-6	346.5	Y	-	199 mg/L @ 25° C	-	-	$47	Steroid	Pharmaceutical

Substance	CASRN[a]	MW[b]	EPA Reference Chemicals	ER Agonist Activity[c]	Solubility in Water[d]	Solubility in DMSO[d]	log K_{ow}[d]	Total Cost Per 500mg[e,f]	MESH Chemical Class[g]	Product Class
Cyproterone acetate	427-51-0	416.9	Y	-	-	-	-	$268	Steroid	Pharmaceutical
Dibenzo[*a*,*h*]anthracene	**53-70-3**	**278.4**	**Y**	**-**	**practically insoluble**	**-**	**6.5**	**$352**	**Polycyclic Compound**	**Laboratory Chemical, Natural Product**
Fluoranthene	**206-44-0**	**202.3**	**Y**	**-**	**0.2 mg/L**	**soluble**	**5.16**	**$21**	**Polycyclic Compound**	**Industrial Chemical, Laboratory Chemical, Pharmaceutical Intermediate**
Fluoxymestrone	76-43-7	336.4	Y	-	practically insoluble	-	2.38	$131	Steroid	Pharmaceutical
Flutamide	13311-84-7	276.2	Y	-	9.45 mg/L @ 25 °C	soluble	3.35	$22	Amide	Pharmaceutical, Veterinary Agent
Linuron	330-55-2	249.1	Y	-	75 mg/L @ 25 °C	-	3.2	$124	Urea	Herbicide
Mifepristone (Mifeprex)	84371-65-3	429.6	Y	-	insoluble	-	-	$262	Steroid	Pharmaceutical
Phenobarbital	**50-06-6**	**232.2**	**Y**	**-**	**1300 mg/L @ 25 °C**	**-**	**1.47**	**$56**	**Heterocyclic Compound, Pyrimidine**	**Pharmaceutical, Veterinary Agent**
Procymidone	32809-16-8	284.1	Y	-	4.5 mg/L @ 25 °C	-	-	$110	Polycyclic Compound	Fungicide

Substance	CASRN[a]	MW[b]	EPA Reference Chemicals	ER Agonist Activity[c]	Solubility in Water[d]	Solubility in DMSO[d]	log K_{ow}[d]	Total Cost Per 500mg[e,f]	MESH Chemical Class[g]	Product Class
Raloxifene[i]	**82640-04-8**	**510.1**	**N**	**-**	**insoluble**	**28 mg/ml**	**-**	**$235**	**Hydrocarbon (Cyclic)**	**Pharmaceutical**
17b-Trenbolone	10161-33-8	270.4	Y	-	-	-	-	$130	Steroid	Pharmaceutical
Vinclozolin	**50471-44-8**	**286.1**	**Y**	**-**	**1000 mg/L @ 20 °C**	**-**	**3.10**	**$41**	**Heterocyclic Compound**	**Fungicide**
Actinomycin D	**50-76-0**	**1255.4**	**Y**	**n.d.[l]**	**1 g/L at 37 ˚C**	**10 mg/ml**	**3.21 @ pH of 7.4**	**$2,285**	**Heterocyclic Compound, Polycyclic Compound**	**Laboratory Chemical, Pharmaceutical, Veterinary Agent**
Ammonium perchlorate	7790-98-9	117.5	N	n.d.	200 g/L at 25 ˚C	-	-	$55	Amine, Onium Compound	Industrial Chemical, Laboratory Chemical, Pharmaceutical
4-Hydroxy androstenedione	566-48-3	302.4	Y	n.d.	-	-	-	$107	Steroid	Pharmaceutical
Apomorphine	58-00-4	267.3	Y	n.d.	1.660 g/mL	-	2.3	$428	Heterocyclic Compound	Pharmaceutical, Veterinary Agent
Bicalutamide	90357-06-5	430.4	Y	n.d.	5 mg/L	-	-	$436	Amide	Pharmaceutical
2-*sec*-Butylphenol	**89-72-5**	**150.2**	**N**	**n.d.**	**insoluble**	**-**	**3.27**	**$16**	**Phenol**	**Chemical Intermediate, Pesticide Intermediate, Plasticizer**

Substance	CASRN[a]	MW[b]	EPA Reference Chemicals	ER Agonist Activity[c]	Solubility in Water[d]	Solubility in DMSO[d]	log K_{ow}[d]	Total Cost Per 500mg[e,f]	MESH Chemical Class[g]	Product Class
Chrysin	480-40-0	254.24	Y	n.d.	84 mg/L @ 25 °C	-	3.52	$60	Flavonoid, Heterocyclic Compound	Natural Product
Clomiphene citrate	**50-41-9**	**598.1**	**Y**	**n.d.**	**slightly soluble**	**-**	**-**	**$45**	**Amine, Carboxylic Acid, Heterocyclic Compound**	**Pharmaceutical**
Cycloheximide	66-81-9	281.4	N	n.d.	0.00021 mg/L @ 25 ˚C	-	0.55	$45	Heterocyclic Compound	Fungicide, Pharmaceutical, Veterinary Agent
Finasteride	98319-26-7	372.5	Y	n.d.	-	-	-	$377	Steroid	Pharmaceutical
Haloperidol	52-86-8	375.9	Y	n.d.	1.4 mg/L @ 25 °C	soluble	4.3	$54	Ketone	Pharmaceutical, Veterinary Agent
Hydroxyflutamide	**52806-53-8**	**292.2**	**N**	**n.d.**	**27.5 mg/L @ 25 °C**	**-**	**2.7**	**$2,941**	**Amide**	**Pharmaceutical**
Ketoconazole	65277-42-1	531.4	Y	n.d.	0.087 mg/L @ 25 °C	-	4.35	$380	Heterocyclic Compound	Pharmaceutical
Medroxyprogesterone acetate	71-58-9	386.5	Y	n.d.	practically insoluble	-	-	$105	Steroid	Pharmaceutical
Morin	**480-16-0**	**302.2**	**Y**	**n.d.**	**250 mg/L @ 25 °C**	**-**	**1.54**	**$14**	**Flavonoid, Heterocyclic Compound**	**Dye, Natural Product, Pharmaceutical Intermediate**
Nilutamide	63612-50-0	317.2	Y	n.d.	insoluble	-	-	$45	Heterocyclic Compound, Imidazole	Pharmaceutical

Substance	CASRN[a]	MW[b]	EPA Reference Chemicals	ER Agonist Activity[c]	Solubility in Water[d]	Solubility in DMSO[d]	log K_{ow}[d]	Total Cost Per 500mg[e,f]	MESH Chemical Class[g]	Product Class
Norethynodrel	**68-23-5**	**298.4**	**Y**	**n.d.**	**practically insoluble**	**-**	**3.51**	**$78**	**Steroid**	**Pharmaceutical**
Oxazepam	604-75-1	286.7	Y	n.d.	179 mg/L	-	2.24	$463	Heterocyclic Compound	Pharmaceutical, Veterinary Agent
Ethyl paraben	**120-47-8**	**166.2**	**Y**	**n.d.**	**885 mg/L @ 25 °C**	**-**	**2.47**	**$8**	**Carboxylic Acid, Phenol**	**Pharmaceutical, Preservative**
Phenolphthalin	**81-90-3**	**320.3**	**Y**	**n.d.**	**175 mg/L @ 20 °C**	**-**	**3.95**	**$26**	**Carboxylic Acid, Phenol**	**Dye, Laboratory Chemical**
Pimozide	2062-78-4	461.6	Y	n.d.	insoluble	18 mg/mL	-	$45	Heterocyclic Compound	Pharmaceutical
Propylthiouracil	**51-52-5**	**170.2**	**Y**	**n.d.**	**1204 mg/L @ 25 °C**	**-**	**-**	**$28**	**Heterocyclic Compound, Pyrimidine**	**Pharmaceutical, Veterinary Agent**
Reserpine	50-55-5	608.7	Y	n.d.	73 mg/L @ 30 °C	-	-	$76	Heterocyclic Compound, Indole	Pharmaceutical, Veterinary Agent
Sodium azide	**26628-22-8**	**65.0**	**Y**	**n.d.**	**41 g/100 mL @ 15 °C**	**-**	**-**	**$17**	**Azide, Salt (inorganic)**	**Chemical Intermediate, Fungicide, Herbicide**
Spironolactone	52-01-7	416.6	Y	n.d.	22 mg/L @ 25 °C	soluble	2.78	$38	Lactone, Steroid	Pharmaceutical
12-*O*-Tetradecanoylphorbol-13-acetate	**16561-29-8**	**616.8**	**N**	**n.d.**	**-**	**soluble**	**-**	**$11,200**	**Hydrocarbon (Cyclic)**	**Laboratory Chemical**
L-Thyroxine	51-48-9	776.9	Y	n.d.	slightly soluble	-	-	$65	Amino Acid	Pharmaceutical, Veterinary Agent

Substances listed in bolded text are included on the ICCVAM Minimum List of Substances for Validation of ER binding and TA Assays.
**p,p*'-DDE =1,1-Dichloro-2,2-di(*p*-chlorophenyl)ethylene; *o,p*'-DDT =1,1,1-Trichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)ethane
[a] CASRN = Chemical Abstracts Service Registry Number
[b] MW = Molecular Weight
[c] +++ Indicates that the substance was strongly active (EC_{50} value was <0.001 □M); ++ indicates that the substance was moderately active (EC_{50} value was between 0.001 and 0.1 □M); + indicates that the substance was weakly active (EC_{50} value was >0.1 □M), or a positive response was reported without an EC50 value. The EC_{50} is the effective concentration that causes half-maximal activation of the receptor.
[d] Information on Solubility in Water, Solubility in DMSO, and log $K_{ow,}$ were obtained from the National Library of Medicine's ChemIDplus http://chem.sis.nlm.nih.gov/chemidplus/, and from manufacturer Materials Safety Data Sheets (MSDSs).
[e] 500 mg is the expected minimum amount of substance required per laboratory to conduct an endocrine disruptor (ED) validation study.
[f] Pricing information was obtained from vendors during October of 2005 and reflects the cost of 500 mg of substance, or the minimum amount sold.
[g] MeSH = Medical Subject Headings, information on chemical class criteria can be obtained at www.nlm.nih.gov/MeSH
[h] A "-" in the fields for Solubility in Water, Solubility in DMSO or low K_{ow} indicates that there is no data for this field.
[i] Raloxifene may act as an agonist in some *in vitro* systems.
[j] N indicates that this substance is not included on the EPA reference chemical list.
[k] Y indicates that this substance is included on the EPA reference chemical list.
[l] n.d. indicates that no relevant data were identified.

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Appendix B-3

Revised ICCVAM List of Reference Substances for Validation of *In Vitro* ER TA Antagonist Assays (Sorted by ER Antagonism and Substance Name)

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Appendix B-3 Revised ICCVAM List of Reference Substances for Validation of *In Vitro* ER TA Antagonist Assays (Sorted by ER TA Antagonism and Substance Name)

Substance	CASRN[a]	MW[b]	EPA Reference Chemicals	ER Antagonism[c]	Solubility in Water[d]	Solubility in DMSO[d]	log Kow[d]	Total Cost Per 500mg[e,f]	MESH Chemical Class[g]	Product Class
Flavone	**525-82-6**	**222.2**	**N[j]**	**###**	**0.1 mg/L @ 25° C**	**-[h]**	**5.08**	**$48**	**Flavonoid, Heterocyclic Compound**	**Natural Product, Pharmaceutical**
Raloxifene[i]	**82640-04-8**	**510.1**	**N**	**###**	**insoluble**	**28 mg/ml**	**-**	**$235**	**Hydrocarbon (Cyclic)**	**Pharmaceutical**
Tamoxifen	**10540-29-1**	**371.5**	**Y[k]**	**###**	**practically insoluble**	**soluble**	**-**	**$125**	**Hydrocarbon (Cyclic)**	**Pharmaceutical**
4-Hydroxytamoxifen	**68047-06-3**	**387.5**	**N**	**###**	**practically insoluble**	**soluble**	**-**	**$6,090**	**Hydrocarbon (Cyclic)**	**Pharmaceutical**
Dibenzo[*a*,*h*]anthracene	**53-70-3**	**278.4**	**Y**	**##**	**practically insoluble**	**-**	**6.5**	**$352**	**Polycyclic Compound**	**Laboratory Chemical, Natural Product**
o*,*p'* –DDT	**789-02-6**	**354.5**	**Y**	**#**	**0.085 mg/L @ 25° C**	**-**	**6.79**	**$714**	**Hydrocarbon (Halogenated)**	**Pesticide**
Fenarimol	60168-88-9	331.2	Y	#	14 mg/L @ 25° C	-	3.6	$375	Heterocyclic Compound, Pyrimidine	Fungicide
***p* -n -Nonylphenol**	**104-40-5**	**220.4**	**Y**	**#**	**-**	**-**	**5.76**	**$192**	**Phenol**	**Chemical Intermediate**
Resveratrol	**501-36-0**	**228.2**	**N**	**#**	**-**	**-**	**3.08**	**$226**	**Hydrocarbon (Cyclic)**	**Natural Product**

Substance	CASRN[a]	MW[b]	EPA Reference Chemicals	ER Antagonism[c]	Solubility in Water[d]	Solubility in DMSO[d]	log Kow[d]	Total Cost Per 500mg[e,f]	MESH Chemical Class[g]	Product Class
Genistein	**446-72-0**	**270.2**	**Y**	**#**	**insoluble**	**50 mg/ml @ 25˚ C**	**2.84**	**$943**	**Flavonoid, Heterocyclic Compound**	**Natural Product, Pharmaceutical**
Apigenin	**520-36-5**	**270.2**	**N**	**#-**	**183 mg/L @ 25˚ C**	**27 mg/ml @ 25˚ C**	**-**	**$790**	**Heterocyclic Compound**	**Dye, Natural Product, Pharmaceutical Intermediate**
Atrazine	**1912-24-9**	**215.7**	**Y**	**-**	**34.7 mg/L @ 26˚ C**	**183 g/kg @ 25˚ C**	**2.61**	**$68**	**Heterocyclic Compound**	**Herbicide**
Bisphenol A	**80-05-7**	**228.3**	**Y**	**-**	**120 mg/L @ 25˚ C**	**-**	**3.32**	**$12**	**Phenol**	**Chemical Intermediate, Flame Retardant, Fungicide**
Corticosterone	**50-22-6**	**346.5**	**Y**	**-**	**199 mg/L @ 25˚ C**	**-**	**-**	**$47**	**Steroid**	**Pharmaceutical**
Coumestrol	**479-13-0**	**268.2**	**Y**	**-**	**practically insoluble**	**-**	**-**	**$1,550**	**Heterocyclic Compound**	**Natural Product**
Daidzein	**486-66-8**	**254.2**	**Y**	**-**	**practically insoluble**	**10 mg/ml**	**-**	**$735**	**Flavonoid, Heterocyclic Compound**	**Natural Product**
p,p'* –DDE	**72-55-9**	**318.0**	**Y**	**-**	**0.04 mg/L 25 °C**	**-**	**6.51**	**$94**	**Hydrocarbon (Halogenated)**	**Pesticide Intermediate**
Dicofol	**115-32-2**	**370.489**	**Y**	**-**	**1.2 mg/L @24°C**	**-**	**4.28**	**$88**	**Hydrocarbon (Cyclic), Hydrocarbon (Halogenated)**	**Pesticide**

Substance	CASRN[a]	MW[b]	EPA Reference Chemicals	ER Antagonism[c]	Solubility in Water[d]	Solubility in DMSO[d]	log Kow[d]	Total Cost Per 500mg[e,f]	MESH Chemical Class[g]	Product Class
Diethylstilbestrol	**56-53-1**	**268.4**	**Y**	**-**	**12 mg/L @ 25° C**	**-**	**5.07**	**$47**	**Hydrocarbon (Cyclic)**	**Pharmaceutical, Veterinary Agent**
17a-Ethinyl estradiol	**57-63-6**	**296.4**	**Y**	**-**	**insoluble**	**-**	**3.67**	**$35**	**Steroid**	**Pharmaceutical, Veterinary Agent**
Estrone	**53-16-7**	**270.4**	**Y**	**-**	**0.003 g/ 100 mL @ 25° C**	**-**	**3.13**	**$14**	**Steroid**	**Pharmaceutical, Veterinary Agent**
Fluoranthene	**206-44-0**	**202.3**	**Y**	**-**	**0.2 mg/L**	**soluble**	**5.16**	**$21**	**Polycyclic Compound**	**Industrial Chemical, Laboratory Chemical, Pharmaceutical Intermediate**
Fluoxymestrone	76-43-7	336.4	Y	-	practically insoluble	-	2.38	$131	Steroid	Pharmaceutical
Kaempferol	**520-18-3**	**286.2**	**Y**	**-**	**slightly soluble**	**-**	**1.96**	**$390**	**Flavonoid, Heterocyclic Compound**	**Natural Product**
***p,p'*- Methoxychlor**	**72-43-5**	**345.7**	**Y**	**-**	**30.5 mg/L @ 25 °C**	**-**	**5.08**	**$17**	**Hydrocarbon (Halogenated)**	**Pesticide, Veterinary Agent**
Butylbenzyl phthalate	**85-68-7**	**312.4**	**Y**	**-**	**2.69 mg/L @ 25° C**	**-**	**4.91**	**$79**	**Carboxylic Acid, Phthalic Acid**	**Chemical Intermediate, Plasticizer**
Di - *n* -butyl phthalate	**84-74-2**	**278.3**	**Y**	**-**	**-**	**-**	**-**	**$34**	**Ester, Phthalic Acid**	**Cosmetic Ingredient, Industrial Chemical, Plasticizer**

Substance	CASRN[a]	MW[b]	EPA Reference Chemicals	ER Antagonism[c]	Solubility in Water[d]	Solubility in DMSO[d]	log Kow[d]	Total Cost Per 500mg[e,f]	MESH Chemical Class[g]	Product Class
Progesterone	**57-83-0**	**314.5**	**Y**	**-**	**8.8 mg/L @ 25 ˚C**	**-**	**3.87**	**$25**	**Steroid**	**Pharmaceutical, Veterinary Agent**
Actinomycin D	**50-76-0**	**1255.4**	**Y**	**n.d.[1]**	**1 g/L at 37 ˚C**	**10 mg/ml**	**3.21 @ pH of 7.4**	**$2,285**	**Heterocyclic Compound, Polycyclic Compound**	**Laboratory Chemical, Pharmaceutical, Veterinary Agent**
Ammonium perchlorate	7790-98-9	117.5	N	n.d.	200 g/L at 25 ˚C	-	-	$55	Amine, Onium Compound	Industrial Chemical, Laboratory Chemical, Pharmaceutical
4-Androstenedione	**63-05-8**	**286.4**	**N**	**n.d.**	**57.8 mg/mL 25 °C**	**-**	**2.75**	**$53**	**Steroid**	Pharmaceutical
4-Hydroxy androstenedione	566-48-3	302.4	Y	n.d.	-	-	-	$107	Steroid	Pharmaceutical
Apomorphine	58-00-4	267.3	Y	n.d.	1.660 g/mL	-	2.3	$428	Heterocyclic Compound	Pharmaceutical, Veterinary Agent
Bicalutamide	90357-06-5	430.4	Y	n.d.	5 mg/L	-	-	$436	Amide	Pharmaceutical

Substance	CASRN[a]	MW[b]	EPA Reference Chemicals	ER Antagonism[c]	Solubility in Water[d]	Solubility in DMSO[d]	log Kow[d]	Total Cost Per 500mg[e,f]	MESH Chemical Class[g]	Product Class
Bisphenol B	**77-40-7**	**242.3**	**Y**	**n.d.**	**1 g in 50 mL**	**-**	**2.30**	**$110**	**Phenol**	**Chemical Intermediate, Flame Retardant, Fungicide**
2-*sec*-Butylphenol	**89-72-5**	**150.2**	**N**	**n.d.**	**insoluble**	**-**	**3.27**	**$16**	**Phenol**	**Chemical Intermediate, Pesticide Intermediate, Plasticizer**
Chrysin	480-40-0	254.24	Y	n.d.	84 mg/L @ 25 °C	-	3.52	$60	Flavonoid, Heterocyclic Compound	Natural Product
Clomiphene citrate	**50-41-9**	**598.1**	**Y**	**n.d.**	**slightly soluble**	**-**	**-**	**$45**	**Amine, Carboxylic Acid, Heterocyclic Compound**	**Pharmaceutical**
12-*O*-Tetradecanoylphorbol-13-acetate	**16561-29-8**	**616.8**	**N**	**n.d.**	**-**	**soluble**	**-**	**$11,200**	**Hydrocarbon (Cyclic)**	**Chemical Intermediate**
4-Cumylphenol	**599-64-4**	**212.3**	**N**	**n.d.**	**insoluble**	**-**	**-**	**$24**	**Phenol**	**Fungicide, Pharmaceutical, Veterinary Agent**
Cycloheximide	66-81-9	281.4	N	n.d.	0.00021 mg/L @ 25 °C	-	0.55	$45	Heterocyclic Compound	Pharmaceutical

Substance	CASRN[a]	MW[b]	EPA Reference Chemicals	ER Antagonism[c]	Solubility in Water[d]	Solubility in DMSO[d]	log Kow[d]	Total Cost Per 500mg[e,f]	MESH Chemical Class[g]	Product Class
Cyproterone acetate	427-51-0	416.9	Y	n.d.	-	-	-	$268	Steroid	Pharmaceutical, Veterinary Agent
Dexamethasone	**50-02-2**	**392.5**	**Y**	**n.d.**	**10 mg/100mL @ 25 °C**	**-**	**-**	**$352**	**Steroid**	**Pharmaceutical**
5a-Dihydrotestosterone	**521-18-6**	**290.4**	**Y**	**n.d.**	**practically insoluble**	**-**	**3.55**	**$27**	**Steroid**	**Pharmaceutical, Veterinary Agent**
17a-Estradiol	**57-91-0**	**272.4**	**Y**	**n.d.**	**3.9 mg/L**	**-**	**3.94**	**$230**	**Steroid**	**Pharmaceutical, Veterinary Agent**
17ß-Estradiol	**50-28-2**	**272.4**	**Y**	**n.d.**	**3.60 mg/L @ 27 °C**	**soluble**	**4.01**	**$151**	**Steroid**	**Pharmaceutical**
Finasteride	98319-26-7	372.5	Y	n.d.	-	-	-	$377	Steroid	Pharmaceutical, Veterinary Agent
Flutamide	13311-84-7	276.2	Y	n.d.	9.45 mg/L @ 25 °C	soluble	3.35	$22	Amide	Pharmaceutical, Veterinary Agent
Haloperidol	52-86-8	375.9	Y	n.d.	1.4 mg/L @ 25 °C	soluble	4.3	$54	Ketone	Pharmaceutical, Veterinary Agent

Substance	CASRN[a]	MW[b]	EPA Reference Chemicals	ER Antagonism[c]	Solubility in Water[d]	Solubility in DMSO[d]	log Kow[d]	Total Cost Per 500mg[e,f]	MESH Chemical Class[g]	Product Class
***meso*-Hexestrol**	**84-16-2**	**270.4**	**Y**	**n.d.**	**-**	**-**	**-**	**$35**	**Steroid**	**Pharmaceutical**
Hydroxyflutamide	**52806-53-8**	**292.2**	**N**	**n.d.**	**27.5 mg/L @ 25 °C**	**-**	**2.7**	**$2,941**	**Amide**	**Pesticide**
Kepone	**143-50-0**	**490.6**	**Y**	**n.d.**	**2.70 mg/L @ 25 °C**	**-**	**5.41**	**$123**	**Hydrocarbon (Halogenated)**	**Pharmaceutical**
Ketoconazole	65277-42-1	531.4	Y	n.d.	0.087 mg/L @ 25 °C	-	4.35	$380	Heterocyclic Compound	Herbicide
Linuron	330-55-2	249.1	Y	n.d.	75 mg/L @ 25 °C	-	3.2	$124	Urea	Pharmaceutical
Medroxyprogesterone acetate	71-58-9	386.5	Y	n.d.	practically insoluble	-	-	$105	Steroid	Pharmaceutical
Mifepristone (Mifeprex)	84371-65-3	429.6	Y	n.d.	insoluble	-	-	$262	Steroid	Dye, Natural Product, Pharmaceutical Intermediate
Morin	**480-16-0**	**302.2**	**Y**	**n.d.**	**250 mg/L @ 25 °C**	**-**	**1.54**	**$14**	**Flavonoid, Heterocyclic Compound**	**Pharmaceutical**
Nilutamide	63612-50-0	317.2	Y	n.d.	insoluble	-	-	$45	Heterocyclic Compound, Imidazole	Pharmaceutical

Substance	CASRN[a]	MW[b]	EPA Reference Chemicals	ER Antagonism[c]	Solubility in Water[d]	Solubility in DMSO[d]	log Kow[d]	Total Cost Per 500mg[e,f]	MESH Chemical Class[g]	Product Class
Norethynodrel	**68-23-5**	**298.4**	**Y**	**n.d.**	**practically insoluble**	**-**	**3.51**	**$78**	**Steroid**	**Pharmaceutical, Veterinary Agent**
19-Nortestosterone	434-22-0	274.4	N	n.d.	-	-	-	$90	Steroid	Chemical Intermediate, Pharmaceutical Intermediate
4-*tert*-Octylphenol	**140-66-9**	**206.3**	**Y**	**n.d.**	**-**	**-**	**-**	**$28**	**Phenol**	**Pharmaceutical, Veterinary Agent**
Oxazepam	604-75-1	286.7	Y	n.d.	179 mg/L	-	2.24	$463	Heterocyclic Compound	Pharmaceutical, Preservative
Ethyl paraben	**120-47-8**	**166.2**	**Y**	**n.d.**	**885 mg/L @ 25 °C**	**-**	**2.47**	**$8**	**Carboxylic Acid, Phenol**	**Pharmaceutical, Veterinary Agent**
Phenobarbital	**50-06-6**	**232.2**	**Y**	**n.d.**	**1300 mg/L @ 25 °C**	**-**	**1.47**	**$56**	**Heterocyclic Compound, Pyrimidine**	**Dye, Laboratory Chemical**
Phenolphthalin	**81-90-3**	**320.3**	**Y**	**n.d.**	**175 mg/L @ 20 °C**	**-**	**3.95**	**$26**	**Carboxylic Acid, Phenol**	**Pesticide Intermediate, Plasticizer**
Diethylhexyl phthalate	**117-81-7**	**330.2**	**Y**	**n.d.**	**0.285 mg/L @ 24 °C**	**-**	**7.6**	**$26**	**Phthalic Acid**	**Pharmaceutical**

Substance	CASRN[a]	MW[b]	EPA Reference Chemicals	ER Antagonism[c]	Solubility in Water[d]	Solubility in DMSO[d]	log Kow[d]	Total Cost Per 500mg[e,f]	MESH Chemical Class[g]	Product Class
Pimozide	2062-78-4	461.6	Y	n.d.	insoluble	18 mg/mL	-	$45	Heterocyclic Compound	Fungicide
Procymidone	32809-16-8	284.1	Y	n.d.	4.5 mg/L @ 25 °C	-	-	$110	Polycyclic Compound	Pharmaceutical, Veterinary Agent
Propylthiouracil	**51-52-5**	**170.2**	**Y**	**n.d.**	**1204 mg/L @ 25 °C**	**-**	**-**	**$28**	**Heterocyclic Compound, Pyrimidine**	**Pharmaceutical, Veterinary Agent**
Reserpine	50-55-5	608.7	Y	n.d.	73 mg/L @ 30 °C	-	-	$76	Heterocyclic Compound, Indole	Chemical Intermediate, Fungicide, Herbicide
Sodium azide	**26628-22-8**	**65.0**	**Y**	**n.d.**	**41 g/100 mL @ 15 °C**	**-**	**-**	**$17**	**Azide, Salt (inorganic)**	**Pharmaceutical**
Spironolactone	52-01-7	416.6	Y	n.d.	22 mg/L @ 25 °C	soluble	2.78	$38	Lactone, Steroid	Pharmaceutical, Veterinary Agent
Testosterone	**58-22-0**	**288.4**	**N**	**n.d.**	**practically insoluble**	**soluble**	**-**	**$26**	**Steroid**	**Pharmaceutical, Veterinary Agent**
Methyl testosterone	**58-18-4**	**302.5**	**Y**	**n.d.**	**-**	**-**	**3.32**	**$26**	**Steroid**	**Laboratory Chemical**

Substance	CASRN[a]	MW[b]	EPA Reference Chemicals	ER Antagonism[c]	Solubility in Water[d]	Solubility in DMSO[d]	log Kow[d]	Total Cost Per 500mg[e,f]	MESH Chemical Class[g]	Product Class
L-Thyroxine	51-48-9	776.9	Y	n.d.	slightly soluble	-	-	$65	Amino Acid	Pharmaceutical, Veterinary Agent
17b-Trenbolone	10161-33-8	270.4	Y	n.d.	-	-	-	$130	Steroid	Pharmaceutical
2,4,5-Trichlorophenoxyacetic acid	**93-76-5**	**255.5**	**Y**	**n.d.**	**278 mg/L**	**-**	**3.31**	**$48**	**Carboxylic Acid**	**Herbicide**
Vinclozolin	**50471-44-8**	**286.1**	**Y**	**n.d.**	**1000 mg/L @ 20 °C**	**-**	**3.10**	**$41**	**Heterocyclic Compound**	**Fungicide**

Substances listed in bolded text are included on the ICCVAM Minimum List of Substances for Validation of ER binding and TA Assays.
*_p,p'_-DDE =1,1-Dichloro-2,2-di(_p_-chlorophenyl)ethylene; _o,p'_-DDT =1,1,1-Trichloro-2-(_o_-chlorophenyl)-2-(_p_-chlorophenyl)ethane
[a] CASRN = Chemical Abstracts Service Registry Number
[b] MW = Molecular Weight
[c] ### Indicates that the substance was uniformly positive in multiple assays; ## indicates that the substance was positive in the majority of assays in which it was tested; # indicates that the substance was positive in the single assay in which it was tested; #- indicates the substance was positive in one assay but was also negative in one or more assays; - indicates that the substance was uniformly negative in multiple assays.
[d] Information on Solubility in Water, Solubility in DMSO, and log $K_{ow,}$ were obtained from the National Library of Medicine's ChemIDplus http://chem.sis.nlm.nih.gov/chemidplus/, and from manufacturer Materials Safety Data Sheets (MSDSs).
[e] 500 mg is the expected minimum amount of substance required per laboratory to conduct an endocrine disruptor (ED) validation study.
[f] Pricing information was obtained from vendors during October of 2005 and reflects the cost of 500 mg of substance, or the minimum amount sold.
[g] MeSH = Medical Subject Headings, information on chemical class criteria can be obtained at www.nlm.nih.gov/MeSH
[h] A "-" in the fields for Solubility in Water, Solubility in DMSO or low K_{ow} indicates that there is no data for this field.
[i] Raloxifene may act as an agonist in some *in vitro* systems.
[j] N indicates that this substance is not included on the EPA reference chemical list.
[k] Y indicates that this substance is included on the EPA reference chemical list.
[l] n.d. indicates that no relevant data were identified.

Appendix B-4

Revised ICCVAM List of Reference Substances for Validation of *In Vitro A*R Binding Assays (Sorted by AR Binding Activity and Substance Name)

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Appendix B-4 Revised ICCVAM List of Reference Substances for Validation of *In Vitro* AR Binding Assays (Sorted by AR Binding Activity and Substance Name)

Substance	CASRN[a]	MW[b]	EPA Reference Chemicals	AR Binding Activity[c]	Solubility in Water[d]	Solubility in DMSO[d]	log K_{ow}[d]	Total Cost Per 500mg[e,f]	MESH Chemical Class[g]	Product Class
4-Androstenedione	63-05-8	286.4	N[j]	+++	57.8 mg/mL 25 °C	-[h]	2.75	$53	Steroid	Pharmaceutical
Bicalutamide	90357-06-5	430.4	Y[k]	+++	5 mg/L	-	-	$436	Amide	Pharmaceutical
Cyproterone acetate	427-51-0	416.9	Y	+++	-	-	-	$268	Steroid	Pharmaceutical
5a-Dihydrotestosterone	521-18-6	290.4	Y	+++	practically insoluble	-	3.55	$27	Steroid	Pharmaceutical
Medroxyprogesterone acetate	71-58-9	386.5	Y	+++	practically insoluble	-	-	$105	Steroid	Pharmaceutical
Mifepristone (Mifeprex)	84371-65-3	429.6	Y	+++	insoluble	-	-	$262	Steroid	Pharmaceutical
Nilutamide	63612-50-0	317.2	Y	+++	insoluble	-	-	$45	Heterocyclic Compound, Imidazole	Pharmaceutical
19-Nortestosterone	434-22-0	274.4	N	+++	-	-	-	$90	Steroid	Pharmaceutical, Veterinary Agent
Progesterone	57-83-0	314.5	Y	+++	8.8 mg/L @ 25 °C	-	3.87	$25	Steroid	Pharmaceutical, Veterinary Agent
Spironolactone	52-01-7	416.6	Y	+++	22 mg/L @ 25 °C	soluble	2.78	$38	Lactone, Steroid	Pharmaceutical

Substance	CASRN[a]	MW[b]	EPA Reference Chemicals	AR Binding Activity[c]	Solubility in Water[d]	Solubility in DMSO[d]	log K_{ow}[d]	Total Cost Per 500mg[e,f]	MESH Chemical Class[g]	Product Class
Testosterone	**58-22-0**	**288.4**	**N**	**+++**	**practically insoluble**	**soluble**	**-**	**$26**	**Steroid**	**Pharmaceutical, Veterinary Agent**
Methyl testosterone	**58-18-4**	**302.5**	**Y**	**+++**	**-**	**-**	**3.32**	**$26**	**Steroid**	**Pharmaceutical, Veterinary Agent**
17b-Trenbolone	**10161-33-8**	**270.4**	**Y**	**+++**	**-**	**-**	**-**	**$130**	**Steroid**	**Pharmaceutical**
4-Hydroxy androstenedione	566-48-3	302.4	Y	++	-	-	-	$107	Steroid	Pharmaceutical
p,p'* –DDE	**72-55-9**	**318.0**	**Y**	**++**	**0.04 mg/L 25 °C**	**-**	**6.51**	**$94**	**Hydrocarbon (Halogenated)**	**Pesticide Intermediate**
Diethylstilbestrol	**56-53-1**	**268.4**	**Y**	**++**	**12 mg/L @ 25° C**	**-**	**5.07**	**$47**	**Hydrocarbon (Cyclic)**	**Pharmaceutical, Veterinary Agent**
17a-Ethinyl estradiol	**57-63-6**	**296.4**	**Y**	**++**	**insoluble**	**-**	**3.67**	**$35**	**Steroid**	**Pharmaceutical, Veterinary Agent**
17ß-Estradiol	**50-28-2**	**272.4**	**Y**	**++**	**3.60 mg/L @ 27 °C**	**soluble**	**4.01**	**$151**	**Steroid**	**Pharmaceutical, Veterinary Agent**
Estrone	**53-16-7**	**270.4**	**Y**	**++**	**0.003 g/ 100 mL @ 25° C**	**-**	**3.13**	**$14**	**Steroid**	**Pharmaceutical, Veterinary Agent**
Fluoxymestrone	**76-43-7**	**336.4**	**Y**	**++**	**practically insoluble**	**-**	**2.38**	**$131**	**Steroid**	**Pharmaceutical**

Substance	CASRN[a]	MW[b]	EPA Reference Chemicals	AR Binding Activity[c]	Solubility in Water[d]	Solubility in DMSO[d]	log K_{ow}[d]	Total Cost Per 500mg[e,f]	MESH Chemical Class[g]	Product Class
Flutamide	13311-84-7	276.2	Y	++	9.45 mg/L @ 25 °C	soluble	3.35	$22	Amide	Pharmaceutical, Veterinary Agent
Hydroxyflutamide	**52806-53-8**	**292.2**	**N**	++	**27.5 mg/L @ 25 °C**	-	**2.7**	**$2,941**	**Amide**	**Pharmaceutical**
Kepone	**143-50-0**	**490.6**	**Y**	++	**2.70 mg/L @ 25 °C**	-	**5.41**	**$123**	**Hydrocarbon (Halogenated)**	**Pesticide**
Atrazine	**1912-24-9**	**215.7**	**Y**	+	**34.7 mg/L @ 26° C**	**183 g/kg @ 25° C**	**2.61**	**$68**	**Heterocyclic Compound**	**Herbicide**
Corticosterone	**50-22-6**	**346.5**	**Y**	+	**199 mg/L @ 25° C**	-	-	**$47**	**Steroid**	**Pharmaceutical**
o,p'*–DDT	**789-02-6**	**354.5**	**Y**	+	**0.085 mg/L @ 25° C**	-	**6.79**	**$714**	**Hydrocarbon (Halogenated)**	**Pesticide**
Linuron	**330-55-2**	**249.1**	**Y**	+	**75 mg/L @ 25 °C**	-	**3.2**	**$124**	**Urea**	**Herbicide**
***p,p'*- Methoxychlor**	**72-43-5**	**345.7**	**Y**	+	**30.5 mg/L @ 25 °C**	-	**5.08**	**$17**	**Hydrocarbon (Halogenated)**	**Pesticide, Veterinary Agent**
Procymidone	**32809-16-8**	**284.1**	**Y**	+	**4.5 mg/L @ 25 °C**	-	-	**$110**	**Polycyclic Compound**	**Fungicide**
Dexamethasone	**50-02-2**	**392.5**	**Y**	-	**10 mg/100mL @ 25 °C**	-	-	**$352**	**Steroid**	**Pharmaceutical, Veterinary Agent**

Substance	CASRN[a]	MW[b]	EPA Reference Chemicals	AR Binding Activity[c]	Solubility in Water[d]	Solubility in DMSO[d]	log K_{ow}[d]	Total Cost Per 500mg[e,f]	MESH Chemical Class[g]	Product Class
Actinomycin D	**50-76-0**	**1255.4**	**Y**	**n.d.[1]**	**1 g/L at 37 ˚C**	**10 mg/ml**	**3.21 @ pH of 7.4**	**$2,285**	**Heterocyclic Compound, Polycyclic Compound**	**Laboratory Chemical, Pharmaceutical, Veterinary Agent**
Ammonium perchlorate	7790-98-9	117.5	N	n.d.	200 g/L at 25 ˚C	-	-	$55	Amine, Onium Compound	Industrial Chemical, Laboratory Chemical, Pharmaceutical
Apigenin	520-36-5	270.2	N	n.d.	183 mg/L @ 25˚ C	27 mg/ml @ 25˚ C	-	$790	Heterocyclic Compound	Dye, Natural Product, Pharmaceutical Intermediate
Apomorphine	58-00-4	267.3	Y	n.d.	1.660 g/mL	-	2.3	$428	Heterocyclic Compound	Pharmaceutical, Veterinary Agent
Bisphenol A	**80-05-7**	**228.3**	**Y**	**n.d.**	**120 mg/L @ 25˚ C**	-	**3.32**	**$12**	**Phenol**	**Chemical Intermediate, Flame Retardant, Fungicide**
Bisphenol B	77-40-7	242.3	Y	n.d.	1 g in 50 mL	-	2.30	$110	Phenol	Chemical Intermediate, Flame Retardant, Fungicide

Substance	CASRN[a]	MW[b]	EPA Reference Chemicals	AR Binding Activity[c]	Solubility in Water[d]	Solubility in DMSO[d]	log K_{ow}[d]	Total Cost Per 500mg[e,f]	MESH Chemical Class[g]	Product Class
2-*sec*-Butylphenol	89-72-5	150.2	N	n.d.	insoluble	-	3.27	$16	Phenol	Chemical Intermediate, Pesticide Intermediate, Plasticizer
Chrysin	480-40-0	254.24	Y	n.d.	84 mg/L @ 25 °C	-	3.52	$60	Flavonoid, Heterocyclic Compound	Natural Product
Clomiphene citrate	50-41-9	598.1	Y	n.d.	slightly soluble	-	-	$45	Amine, Carboxylic Acid, Heterocyclic Compound	Pharmaceutical
Coumestrol	479-13-0	268.2	Y	n.d.	practically insoluble	-	-	$1,550	Heterocyclic Compound	Natural Product
4-Cumylphenol	599-64-4	212.3	N	n.d.	insoluble	-	-	$24	Phenol	Chemical Intermediate
Cycloheximide	66-81-9	281.4	N	n.d.	0.00021 mg/L @ 25 ˚C	-	0.55	$45	Heterocyclic Compound	Fungicide, Pharmaceutical, Veterinary Agent
Daidzein	486-66-8	254.2	Y	n.d.	practically insoluble	10 mg/ml	-	$735	Flavonoid, Heterocyclic Compound	Natural Product

Substance	CASRN[a]	MW[b]	EPA Reference Chemicals	AR Binding Activity[c]	Solubility in Water[d]	Solubility in DMSO[d]	log K_{ow}[d]	Total Cost Per 500mg[e,f]	MESH Chemical Class[g]	Product Class
Dibenzo[*a,h*]anthracene	53-70-3	278.4	Y	n.d.	practically insoluble	-	6.5	$352	Polycyclic Compound	Laboratory Chemical, Natural Product
Dicofol	**115-32-2**	**370.489**	**Y**	**n.d.**	**1.2 mg/L @24°C**	**-**	**4.28**	**$88**	**Hydrocarbon (Cyclic), Hydrocarbon (Halogenated)**	**Pesticide**
17a-Estradiol	57-91-0	272.4	Y	n.d.	3.9 mg/L	-	3.94	$230	Steroid	Pharmaceutical, Veterinary Agent
Fenarimol	60168-88-9	331.2	Y	n.d.	14 mg/L @ 25˚ C	-	3.6	$375	Heterocyclic Compound, Pyrimidine	Fungicide
Finasteride	**98319-26-7**	**372.5**	**Y**	**n.d.**	**-**	**-**	**-**	**$377**	**Steroid**	**Pharmaceutical**
Flavone	525-82-6	222.2	N	n.d.	0.1 mg/L @ 25˚ C	-	5.08	$48	Flavonoid, Heterocyclic Compound	Natural Product, Pharmaceutical
Fluoranthene	**206-44-0**	**202.3**	**Y**	**n.d.**	**0.2 mg/L**	**soluble**	**5.16**	**$21**	**Polycyclic Compound**	**Industrial Chemical, Laboratory Chemical, Pharmaceutical Intermediate**

Substance	CASRN[a]	MW[b]	EPA Reference Chemicals	AR Binding Activity[c]	Solubility in Water[d]	Solubility in DMSO[d]	log K_{ow}[d]	Total Cost Per 500mg[e,f]	MESH Chemical Class[g]	Product Class
Genistein	446-72-0	270.2	Y	n.d.	insoluble	50 mg/ml @ 25˚ C	2.84	$943	Flavonoid, Heterocyclic Compound	Natural Product, Pharmaceutical
Haloperidol	52-86-8	375.9	Y	n.d.	1.4 mg/L @ 25 °C	soluble	4.3	$54	Ketone	Pharmaceutical, Veterinary Agent
meso-Hexestrol	84-16-2	270.4	Y	n.d.	-	-	-	$35	Steroid	Pharmaceutical, Veterinary Agent
4-Hydroxytamoxifen	**68047-06-3**	**387.5**	**N**	**n.d.**	**practically insoluble**	**soluble**	-	**$6,090**	**Hydrocarbon (Cyclic)**	**Pharmaceutical**
Kaempferol	520-18-3	286.2	Y	n.d.	slightly soluble	-	1.96	$390	Flavonoid, Heterocyclic Compound	Natural Product
Ketoconazole	**65277-42-1**	**531.4**	**Y**	**n.d.**	**0.087 mg/L @ 25 °C**	-	**4.35**	**$380**	**Heterocyclic Compound**	**Pharmaceutical**
Morin	480-16-0	302.2	Y	n.d.	250 mg/L @ 25 °C	-	1.54	$14	Flavonoid, Heterocyclic Compound	Dye, Natural Product, Pharmaceutical Intermediate
***p* -n -Nonylphenol**	**104-40-5**	**220.4**	**Y**	**n.d.**	-	-	**5.76**	**$192**	**Phenol**	**Chemical Intermediate**
Norethynodrel	68-23-5	298.4	Y	n.d.	practically insoluble	-	3.51	$78	Steroid	Pharmaceutical

Substance	CASRN[a]	MW[b]	EPA Reference Chemicals	AR Binding Activity[c]	Solubility in Water[d]	Solubility in DMSO[d]	log K_{ow}[d]	Total Cost Per 500mg[e,f]	MESH Chemical Class[g]	Product Class
4-*tert*-Octylphenol	**140-66-9**	**206.3**	**Y**	**n.d.**	**-**	**-**	**-**	**$28**	**Phenol**	**Chemical Intermediate, Pharmaceutical Intermediate**
Oxazepam	604-75-1	286.7	Y	n.d.	179 mg/L	-	2.24	$463	Heterocyclic Compound	Pharmaceutical, Veterinary Agent
Ethyl paraben	120-47-8	166.2	Y	n.d.	885 mg/L @ 25 °C	-	2.47	$8	Carboxylic Acid, Phenol	Pharmaceutical, Preservative
Phenobarbital	**50-06-6**	**232.2**	**Y**	**n.d.**	**1300 mg/L @ 25 °C**	**-**	**1.47**	**$56**	**Heterocyclic Compound, Pyrimidine**	**Pharmaceutical, Veterinary Agent**
Phenolphthalin	81-90-3	320.3	Y	n.d.	175 mg/L @ 20 °C	-	3.95	$26	Carboxylic Acid, Phenol	Dye, Laboratory Chemical
Butylbenzyl phthalate	85-68-7	312.4	Y	n.d.	2.69 mg/L @ 25˚ C	-	4.91	$79	Carboxylic Acid, Phthalic Acid	Chemical Intermediate, Plasticizer
Diethylhexyl phthalate	**117-81-7**	**330.2**	**Y**	**n.d.**	**0.285 mg/L @ 24 °C**	**-**	**7.6**	**$26**	**Phthalic Acid**	**Pesticide Intermediate, Plasticizer**
Di - *n* -butyl phthalate	**84-74-2**	**278.3**	**Y**	**n.d.**	**-**	**-**	**-**	**$34**	**Ester, Phthalic Acid**	**Cosmetic Ingredient, Industrial Chemical, Plasticizer**

Substance	CASRN[a]	MW[b]	EPA Reference Chemicals	AR Binding Activity[c]	Solubility in Water[d]	Solubility in DMSO[d]	log K_{ow}[d]	Total Cost Per 500mg[e,f]	MESH Chemical Class[g]	Product Class
Pimozide	2062-78-4	461.6	Y	n.d.	insoluble	18 mg/mL	-	$45	Heterocyclic Compound	Pharmaceutical
Propylthiouracil	51-52-5	170.2	Y	n.d.	1204 mg/L @ 25 °C	-	-	$28	Heterocyclic Compound, Pyrimidine	Pharmaceutical, Veterinary Agent
Raloxifene[i]	82640-04-8	510.1	N	n.d.	insoluble	28 mg/ml	-	$235	Hydrocarbon (Cyclic)	Pharmaceutical
Reserpine	50-55-5	608.7	Y	n.d.	73 mg/L @ 30 °C	-	-	$76	Heterocyclic Compound, Indole	Pharmaceutical, Veterinary Agent
Resveratrol	501-36-0	228.2	N	n.d.	-	-	3.08	$226	Hydrocarbon (Cyclic)	Natural Product
Sodium azide	**26628-22-8**	**65.0**	**Y**	**n.d.**	**41 g/100 mL @ 15 °C**	-	-	**$17**	**Azide, Salt (inorganic)**	**Chemical Intermediate, Fungicide, Herbicide**
Tamoxifen	10540-29-1	371.5	Y	n.d.	practically insoluble	soluble	-	$125	Hydrocarbon (Cyclic)	Pharmaceutical

Substance	CASRN[a]	MW[b]	EPA Reference Chemicals	AR Binding Activity[c]	Solubility in Water[d]	Solubility in DMSO[d]	log K_{ow}[d]	Total Cost Per 500mg[e,f]	MESH Chemical Class[g]	Product Class
12-*O*-Tetradecanoylphorbol-13-acetate	**16561-29-8**	**616.8**	**-**	**n.d.**	**-**	**soluble**	**-**	**$11,200**	**Hydrocarbon (Cyclic)**	**Laboratory Chemical**
L-Thyroxine	51-48-9	776.9	Y	n.d.	slightly soluble	-	-	$65	Amino Acid	Pharmaceutical, Veterinary Agent
2,4,5-Trichlorophenoxyacetic acid	**93-76-5**	**255.5**	**Y**	**n.d.**	**278 mg/L**	**-**	**3.31**	**$48**	**Carboxylic Acid**	**Herbicide**
Vinclozolin	**50471-44-8**	**286.1**	**Y**	**n.d.**	**1000 mg/L @ 20 °C**	**-**	**3.10**	**$41**	**Heterocyclic Compound**	**Fungicide**

Substances listed in bolded text are included on the ICCVAM Minimum List of Substances for Validation of AR binding and TA Assays.
**p,p'*-DDE =1,1-Dichloro-2,2-di(*p*-chlorophenyl)ethylene; *o,p'*-DDT =1,1,1-Trichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)ethane
[a] CASRN = Chemical Abstracts Service Registry Number
[b] MW = Molecular Weight
[c] +++ Indicates that the substance was strongly active as measured by the relative binding affinity (RBA) (RBA value was >1); ++ indicates that the substance was moderately active (RBA value was between 1 and 0.01); + indicates that the substance was weakly active (RBA value was < than 0.01); - indicates that an IC_{50} value was not obtained and thus an RBA value could not be determined; ± indicates an equivocal response (i.e., in different studies, the substance was reported as positive and negative). The inhibitory concentration 50 (IC_{50}) is the concentration of test substances that displaces 50% of the radiolabeled reference estrogen or androgen from the receptor.
[d] Information on Solubility in Water, Solubility in DMSO, and log $K_{ow,}$ were obtained from the National Library of Medicine's ChemIDplus http://chem.sis.nlm.nih.gov/chemidplus/, and from manufacturer Materials Safety Data Sheets (MSDSs).
[e] 500 mg is the expected minimum amount of substance required per laboratory to conduct an endocrine disruptor (ED) validation study.
[f] Pricing information was obtained from vendors during October of 2005 and reflects the cost of 500 mg of substance, or the minimum amount sold.
[g] MeSH = Medical Subject Headings, information on chemical class criteria can be obtained at www.nlm.nih.gov/MeSH
[h] A "-" in the fields for Solubility in Water, Solubility in DMSO or low K_{ow} indicates that there is no data for this field.
[i] Raloxifene may act as an agonist in some *in vitro* systems.
[j] N indicates that this substance is not included on the EPA reference chemical list.

[k] Y indicates that this substance is included on the EPA reference chemical list.
[l] n.d. indicates that no relevant data were identified.

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Appendix B-5

Revised ICCVAM List of Reference Substances for Validation of *In Vitro A*R TA Agonist Assays (Sorted by AR Agonist Activity and Substance Name)

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Appendix B-5 Revised ICCVAM List of Reference substances for Validation of *In Vitro* AR TA Agonist Assays (Sorted by AR TA Agonist Activity and Substance Name)

Substance	CASRN[a]	MW[b]	EPA Reference Chemicals	AR Agonist Activity[c]	Solubility in Water[d]	Solubility in DMSO[d]	log K_{ow}[d]	Total Cost Per 500mg[e,f]	MESH Chemical Class[g]	Product Class
5α-Dihydrotestosterone	521-18-6	290.4	Y[j]	+++	practically insoluble	-[h]	3.55	$27	Steroid	Pharmaceutical
19-Nortestosterone	434-22-0	274.4	N[k]	+++	-	-	-	$90	Steroid	Pharmaceutical, Veterinary Agent
Testosterone	58-22-0	288.4	N	+++	practically insoluble	soluble	-	$26	Steroid	Pharmaceutical, Veterinary Agent
p,p' –DDE*	72-55-9	318.0	Y	++	0.04 mg/L 25 °C	-	6.51	$94	Hydrocarbon (Halogenated)	Pesticide Intermediate
17ß-Estradiol	50-28-2	272.4	Y	++	3.60 mg/L @ 27 °C	soluble	4.01	$151	Steroid	Pharmaceutical, Veterinary Agent
Estrone	53-16-7	270.4	Y	++	0.003 g/ 100 mL @ 25° C	-	3.13	$14	Steroid	Pharmaceutical, Veterinary Agent
Mifepristone (Mifeprex)	84371-65-3	429.6	Y	++	insoluble	-	-	$262	Steroid	Pharmaceutical
Methyl testosterone	58-18-4	302.5	Y	++	-	-	3.32	$26	Steroid	Pharmaceutical, Veterinary Agent

Substance	CASRN[a]	MW[b]	EPA Reference Chemicals	AR Agonist Activity[c]	Solubility in Water[d]	Solubility in DMSO[d]	log K_{ow}[d]	Total Cost Per 500mg[e,f]	MESH Chemical Class[g]	Product Class
Ketoconazole	**65277-42-1**	**531.4**	**Y**	**+-**	**0.087 mg/L @ 25 °C**	**-**	**4.35**	**$380**	**Heterocyclic Compound**	**Pharmaceutical**
Nilutamide	**63612-50-0**	**317.2**	**Y**	**+-**	**insoluble**	**-**	**-**	**$45**	**Heterocyclic Compound, Imidazole**	**Pharmaceutical**
***p* -n -Nonylphenol**	**104-40-5**	**220.4**	**Y**	**+-**	**-**	**-**	**5.76**	**$192**	**Phenol**	**Chemical Intermediate**
Bicalutamide	**90357-06-5**	**430.4**	**Y**	**+**	**5 mg/L**	**-**	**-**	**$436**	**Amide**	**Pharmaceutical**
Cyproterone acetate	**427-51-0**	**416.9**	**Y**	**+**	**-**	**-**	**-**	**$268**	**Steroid**	**Pharmaceutical**
Dexamethasone	**50-02-2**	**392.5**	**Y**	**+**	**10 mg/100mL @ 25 °C**	**-**	**-**	**$352**	**Steroid**	**Pharmaceutical, Veterinary Agent**
Dibenzo[*a,h*]anthracene	53-70-3	278.4	Y	+	practically insoluble	-	6.5	$352	Polycyclic Compound	Laboratory Chemical, Natural Product
Fluoxymestrone	**76-43-7**	**336.4**	**Y**	**+**	**practically insoluble**	**-**	**2.38**	**$131**	**Steroid**	**Pharmaceutical**
Hydroxyflutamide	**52806-53-8**	**292.2**	**N**	**+**	**27.5 mg/L @ 25 °C**	**-**	**2.7**	**$2,941**	**Amide**	**Pharmaceutical**
Linuron	**330-55-2**	**249.1**	**Y**	**+**	**75 mg/L @ 25 °C**	**-**	**3.2**	**$124**	**Urea**	**Herbicide**
Medroxyprogesterone acetate	**71-58-9**	**386.5**	**Y**	**+**	**practically insoluble**	**-**	**-**	**$105**	**Steroid**	**Pharmaceutical**

Substance	CASRN[a]	MW[b]	EPA Reference Chemicals	AR Agonist Activity[c]	Solubility in Water[d]	Solubility in DMSO[d]	log K_{ow}[d]	Total Cost Per 500mg[e,f]	MESH Chemical Class[g]	Product Class
Progesterone	**57-83-0**	**314.5**	**Y**	+	**8.8 mg/L @ 25 ˚C**	**-**	**3.87**	**$25**	**Steroid**	**Pharmaceutical, Veterinary Agent**
Spironolactone	**52-01-7**	**416.6**	**Y**	+	**22 mg/L @ 25 °C**	**soluble**	**2.78**	**$38**	**Lactone, Steroid**	**Pharmaceutical**
Apigenin	520-36-5	270.2	N	-	183 mg/L @ 25˚ C	27 mg/ml @ 25˚ C	-	$790	Heterocyclic Compound	Dye, Natural Product, Pharmaceutical Intermediate
Atrazine	**1912-24-9**	**215.7**	**Y**	**-**	**34.7 mg/L @ 26˚ C**	**183 g/kg @ 25˚ C**	**2.61**	**$68**	**Heterocyclic Compound**	**Herbicide**
Bisphenol A	**80-05-7**	**228.3**	**Y**	**-**	**120 mg/L @ 25˚ C**	**-**	**3.32**	**$12**	**Phenol**	**Chemical Intermediate, Flame Retardant, Fungicide**
Bisphenol B	77-40-7	242.3	Y	-	1 g in 50 mL	-	2.30	$110	Phenol	Chemical Intermediate, Flame Retardant, Fungicide
Corticosterone	**50-22-6**	**346.5**	**Y**	**-**	**199 mg/L @ 25˚ C**	**-**	**-**	**$47**	**Steroid**	**Pharmaceutical**
Coumestrol	479-13-0	268.2	Y	-	practically insoluble	-	-	$1,550	Heterocyclic Compound	Natural Product
4-Cumylphenol	599-64-4	212.3	N	-	insoluble	-	-	$24	Phenol	Chemical Intermediate

Substance	CASRN[a]	MW[b]	EPA Reference Chemicals	AR Agonist Activity[c]	Solubility in Water[d]	Solubility in DMSO[d]	log K_{ow}[d]	Total Cost Per 500mg[e,f]	MESH Chemical Class[g]	Product Class
o,p'* –DDT	**789-02-6**	**354.5**	**Y**	**-**	**0.085 mg/L @ 25° C**	**-**	**6.79**	**$714**	**Hydrocarbon (Halogenated)**	**Pesticide**
Diethylstilbestrol	**56-53-1**	**268.4**	**Y**	**-**	**12 mg/L @ 25° C**	**-**	**5.07**	**$47**	**Hydrocarbon (Cyclic)**	**Pharmaceutical, Veterinary Agent**
17α-Estradiol	57-91-0	272.4	Y	-	3.9 mg/L	-	3.94	$230	Steroid	Pharmaceutical, Veterinary Agent
17α-Ethinyl estradiol	**57-63-6**	**296.4**	**Y**	**-**	**insoluble**	**-**	**3.67**	**$35**	**Steroid**	**Pharmaceutical, Veterinary Agent**
Finasteride	**98319-26-7**	**372.5**	**Y**	**-**	**-**	**-**	**-**	**$377**	**Steroid**	**Pharmaceutical**
Flutamide	13311-84-7	276.2	Y	-	9.45 mg/L @ 25 °C	soluble	3.35	$22	Amide	Pharmaceutical, Veterinary Agent
4-Hydroxytamoxifen	**68047-06-3**	**387.5**	**N**	**-**	**practically insoluble**	**soluble**	**-**	**$6,090**	**Hydrocarbon (Cyclic)**	**Pharmaceutical**
Kepone	**143-50-0**	**490.6**	**Y**	**-**	**2.70 mg/L @ 25 °C**	**-**	**5.41**	**$123**	**Hydrocarbon (Halogenated)**	**Pesticide**
***p,p'*- Methoxychlor**	**72-43-5**	**345.7**	**Y**	**-**	**30.5 mg/L @ 25 °C**	**-**	**5.08**	**$17**	**Hydrocarbon (Halogenated)**	**Pesticide, Veterinary Agent**

Substance	CASRN[a]	MW[b]	EPA Reference Chemicals	AR Agonist Activity[c]	Solubility in Water[d]	Solubility in DMSO[d]	log K_{ow}[d]	Total Cost Per 500mg[e,f]	MESH Chemical Class[g]	Product Class
4-*tert*-Octylphenol	**140-66-9**	**206.3**	**Y**	**-**	**-**	**-**	**-**	**$28**	**Phenol**	**Chemical Intermediate, Pharmaceutical Intermediate**
Phenobarbital	**50-06-6**	**232.2**	**Y**	**-**	**1300 mg/L @ 25 °C**	**-**	**1.47**	**$56**	**Heterocyclic Compound, Pyrimidine**	**Pharmaceutical, Veterinary Agent**
Butylbenzyl phthalate	85-68-7	312.4	Y	-	2.69 mg/L @ 25° C	-	4.91	$79	Carboxylic Acid, Phthalic Acid	Chemical Intermediate, Plasticizer
Diethylhexyl phthalate	**117-81-7**	**330.2**	**Y**	**-**	**0.285 mg/L @ 24 °C**	**-**	**7.6**	**$26**	**Phthalic Acid**	**Pesticide Intermediate, Plasticizer**
Di - *n* -butyl phthalate	**84-74-2**	**278.3**	**Y**	**-**	**-**	**-**	**-**	**$34**	**Ester, Phthalic Acid**	**Cosmetic Ingredient, Industrial Chemical, Plasticizer**
Procymidone	**32809-16-8**	**284.1**	**Y**	**-**	**4.5 mg/L @ 25 °C**	**-**	**-**	**$110**	**Polycyclic Compound**	**Fungicide**
Tamoxifen	10540-29-1	371.5	Y	-	practically insoluble	soluble	-	$125	Hydrocarbon (Cyclic)	Pharmaceutical

Substance	CASRN[a]	MW[b]	EPA Reference Chemicals	AR Agonist Activity[c]	Solubility in Water[d]	Solubility in DMSO[d]	log K_{ow}[d]	Total Cost Per 500mg[e,f]	MESH Chemical Class[g]	Product Class
Vinclozolin	**50471-44-8**	**286.1**	**Y**	**-**	**1000 mg/L @ 20 °C**	**-**	**3.10**	**$41**	**Heterocyclic Compound**	**Fungicide**
Actinomycin D	**50-76-0**	**1255.4**	**Y**	**n.d.[i]**	**1 g/L at 37 ˚C**	**10 mg/ml**	**3.21 @ pH of 7.4**	**$2,285**	**Heterocyclic Compound, Polycyclic Compound**	**Laboratory Chemical, Pharmaceutical, Veterinary Agent**
Ammonium perchlorate	7790-98-9	117.5	N	n.d.	200 g/L at 25 ˚C	-	-	$55	Amine, Onium Compound	Industrial Chemical, Laboratory Chemical, Pharmaceutical
4-Androstenedione	**63-05-8**	**286.4**	**N**	**n.d.**	**57.8 mg/mL 25 °C**	**-**	**2.75**	**$53**	**Steroid**	**Pharmaceutical**
4-Hydroxy androstenedione	566-48-3	302.4	Y	n.d.	-	-	-	$107	Steroid	Pharmaceutical
Apomorphine	58-00-4	267.3	Y	n.d.	1.660 g/mL	-	2.3	$428	Heterocyclic Compound	Pharmaceutical, Veterinary Agent
2-*sec*-Butylphenol	89-72-5	150.2	N	n.d.	insoluble	-	3.27	$16	Phenol	Chemical Intermediate, Pesticide Intermediate, Plasticizer

Substance	CASRN[a]	MW[b]	EPA Reference Chemicals	AR Agonist Activity[c]	Solubility in Water[d]	Solubility in DMSO[d]	log K_{ow}[d]	Total Cost Per 500mg[e,f]	MESH Chemical Class[g]	Product Class
Chrysin	480-40-0	254.24	Y	n.d.	84 mg/L @ 25 °C	-	3.52	$60	Flavonoid, Heterocyclic Compound	Natural Product
Clomiphene citrate	50-41-9	598.1	Y	n.d.	slightly soluble	-	-	$45	Amine, Carboxylic Acid, Heterocyclic Compound	Pharmaceutical
Cycloheximide	66-81-9	281.4	N	n.d.	0.00021 mg/L @ 25 ˚C	-	0.55	$45	Heterocyclic Compound	Fungicide, Pharmaceutical, Veterinary Agent
Daidzein	486-66-8	254.2	Y	n.d.	practically insoluble	10 mg/ml	-	$735	Flavonoid, Heterocyclic Compound	Natural Product
Dicofol	**115-32-2**	**370.489**	**Y**	**n.d.**	**1.2 mg/L @24°C**	**-**	**4.28**	**$88**	**Hydrocarbon (Cyclic), Hydrocarbon (Halogenated)**	**Pesticide**
Fenarimol	60168-88-9	331.2	Y	n.d.	14 mg/L @ 25˚ C	-	3.6	$375	Heterocyclic Compound, Pyrimidine	Fungicide
Flavone	525-82-6	222.2	N	n.d.	0.1 mg/L @ 25˚ C	-	5.08	$48	Flavonoid, Heterocyclic Compound	Natural Product, Pharmaceutical
Fluoranthene	**206-44-0**	**202.3**	**Y**	**n.d.**	**0.2 mg/L**	**soluble**	**5.16**	**$21**	**Polycyclic Compound**	**Industrial Chemical, Laboratory Chemical, Pharmaceutical Intermediate**

Substance	CASRN[a]	MW[b]	EPA Reference Chemicals	AR Agonist Activity[c]	Solubility in Water[d]	Solubility in DMSO[d]	log K_{ow}[d]	Total Cost Per 500mg[e,f]	MESH Chemical Class[g]	Product Class
Genistein	446-72-0	270.2	Y	n.d.	insoluble	50 mg/ml @ 25° C	2.84	$943	Flavonoid, Heterocyclic Compound	Natural Product, Pharmaceutical
Haloperidol	52-86-8	375.9	Y	n.d.	1.4 mg/L @ 25 °C	soluble	4.3	$54	Ketone	Pharmaceutical, Veterinary Agent
meso-Hexestrol	84-16-2	270.4	Y	n.d.	-	-	-	$35	Steroid	Pharmaceutical, Veterinary Agent
Kaempferol	520-18-3	286.2	Y	n.d.	slightly soluble	-	1.96	$390	Flavonoid, Heterocyclic Compound	Natural Product
Morin	480-16-0	302.2	Y	n.d.	250 mg/L @ 25 °C	-	1.54	$14	Flavonoid, Heterocyclic Compound	Dye, Natural Product, Pharmaceutical Intermediate
Norethynodrel	68-23-5	298.4	Y	n.d.	practically insoluble	-	3.51	$78	Steroid	Pharmaceutical
Oxazepam	604-75-1	286.7	Y	n.d.	179 mg/L	-	2.24	$463	Heterocyclic Compound	Pharmaceutical, Veterinary Agent
Ethyl paraben	120-47-8	166.2	Y	n.d.	885 mg/L @ 25 °C	-	2.47	$8	Carboxylic Acid, Phenol	Pharmaceutical, Preservative
Phenolphthalin	81-90-3	320.3	Y	n.d.	175 mg/L @ 20 °C	-	3.95	$26	Carboxylic Acid, Phenol	Dye, Laboratory Chemical
Pimozide	2062-78-4	461.6	Y	n.d.	insoluble	18 mg/mL	-	$45	Heterocyclic Compound	Pharmaceutical

Substance	CASRN[a]	MW[b]	EPA Reference Chemicals	AR Agonist Activity[c]	Solubility in Water[d]	Solubility in DMSO[d]	log K_{ow}[d]	Total Cost Per 500mg[e,f]	MESH Chemical Class[g]	Product Class
Propylthiouracil	51-52-5	170.2	Y	n.d.	1204 mg/L @ 25 °C	-	-	$28	Heterocyclic Compound, Pyrimidine	Pharmaceutical, Veterinary Agent
Raloxifene[i]	82640-04-8	510.1	N	n.d.	insoluble	28 mg/ml	-	$235	Hydrocarbon (Cyclic)	Pharmaceutical
Reserpine	50-55-5	608.7	Y	n.d.	73 mg/L @ 30 °C	-	-	$76	Heterocyclic Compound, Indole	Pharmaceutical, Veterinary Agent
Resveratrol	501-36-0	228.2	N	n.d.	-	-	3.08	$226	Hydrocarbon (Cyclic)	Natural Product
Sodium azide	**26628-22-8**	**65.0**	**Y**	**n.d.**	**41 g/100 mL @ 15 °C**	**-**	**-**	**$17**	**Azide, Salt (inorganic)**	**Chemical Intermediate, Fungicide, Herbicide**
12-*O*-Tetradecanoylphorbol-13-acetate	**16561-29-8**	**616.8**	**-**	**n.d.**	**-**	**soluble**	**-**	**$11,200**	**Hydrocarbon (Cyclic)**	**Laboratory Chemical**
L-Thyroxine	51-48-9	776.9	Y	n.d.	slightly soluble	-	-	$65	Amino Acid	Pharmaceutical, Veterinary Agent
17b-Trenbolone	**10161-33-8**	**270.4**	**Y**	**n.d.**	**-**	**-**	**-**	**$130**	**Steroid**	**Pharmaceutical**

Substance	CASRN[a]	MW[b]	EPA Reference Chemicals	AR Agonist Activity[c]	Solubility in Water[d]	Solubility in DMSO[d]	log K_{ow}[d]	Total Cost Per 500mg[e,f]	MESH Chemical Class[g]	Product Class
2,4,5-Trichlorophenoxyacetic acid	**93-76-5**	**255.5**	**Y**	**n.d.**	**278 mg/L**	**-**	**3.31**	**$48**	**Carboxylic Acid**	**Herbicide**

Substances listed in bolded text are included on the ICCVAM Minimum List of Substances for Validation of AR binding and TA Assays.
**p,p'*-DDE =1,1-Dichloro-2,2-di(*p*-chlorophenyl)ethylene; *o,p'*-DDT =1,1,1-Trichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)ethane
[a] CASRN = Chemical Abstracts Service Registry Number
[b] MW = Molecular Weight
[c] +++ Indicates that the substance was strongly active (EC_{50} value was <0.001 □M); ++ indicates that the substance was moderately active (EC_{50} value was between 0.001 and 0.1 □M); + indicates that the substance was weakly active (EC_{50} value was >0.1 □M), or a positive response was reported without an EC_{50} value. The EC_{50} is the effective concentration that causes half-maximal activation of the receptor.
[d] Information on Solubility in Water, Solubility in DMSO, and log $K_{ow,}$ were obtained from the National Library of Medicine's ChemIDplus http://chem.sis.nlm.nih.gov/chemidplus/, and from manufacturer Materials Safety Data Sheets (MSDSs).
[e] 500 mg is the expected minimum amount of substance required per laboratory to conduct an endocrine disruptor (ED) validation study.
[f] Pricing information was obtained from vendors during October of 2005 and reflects the cost of 500 mg of substance, or the minimum amount sold.
[g] MeSH = Medical Subject Headings, information on chemical class criteria can be obtained at www.nlm.nih.gov/MeSH
[h] A "-" in the fields for Solubility in Water, Solubility in DMSO or low K_{ow} indicates that there is no data for this field.
[i] Raloxifene may act as an agonist in some *in vitro* systems.
[j] Y indicates that this substance is included on the EPA reference chemical list.
[k] N indicates that this substance is not included on the EPA reference chemical list.
[l] n.d. indicates that no relevant data were identified.

Appendix B-6

Revised ICCVAM List of Reference Substances for Validation of *In Vitro A*R TA Antagonist Assays (Sorted by AR Antagonism and Substance Name)

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Appendix B-6 Revised ICCVAM List of Reference Substances for Validation of *In Vitro* AR TA Antagonist Assays (Sorted by AR TA Antagonism and Substance Name)

Substance	CASRN[a]	MW[b]	EPA Reference Chemicals	AR Antagonism[c]	Solubility in Water[d]	Solubility in DMSO	log K_{ow}[d]	Total Cost Per 500mg[e,f]	MESH Chemical Class[g]	Product Class
p -n -Nonylphenol	104-40-5	220.4	Y[j]	###	-	-[h]	5.76	$192	Phenol	Chemical Intermediate
Vinclozolin	50471-44-8	286.1	Y	###	1000 mg/L @ 20 °C	-	3.10	$41	Heterocyclic Compound	Fungicide
Bicalutamide	90357-06-5	430.4	Y	##	5 mg/L	-	-	$436	Amide	Pharmaceutical
Cyproterone acetate	427-51-0	416.9	Y	##	-	-	-	$268	Steroid	Pharmaceutical
Hydroxyflutamide	52806-53-8	292.2	N[k]	##	27.5 mg/L @ 25 °C	-	2.7	$2,941	Amide	Pharmaceutical
Nilutamide	63612-50-0	317.2	Y	##	insoluble	-	-	$45	Heterocyclic Compound, Imidazole	Pharmaceutical
Spironolactone	52-01-7	416.6	Y	##	22 mg/L @ 25 °C	soluble	2.78	$38	Lactone, Steroid	Pharmaceutical

Substance	CASRN[a]	MW[b]	EPA Reference Chemicals	AR Antagonism[c]	Solubility in Water[d]	Solubility in DMSO	log K_{ow}[d]	Total Cost Per 500mg[e,f]	MESH Chemical Class[g]	Product Class
Bisphenol A	**80-05-7**	**228.3**	**Y**	**#-**	**120 mg/L @ 25˚ C**	**-**	**3.32**	**$12**	**Phenol**	**Chemical Intermediate, Flame Retardant, Fungicide**
p,p'* –DDE	**72-55-9**	**318.0**	**Y**	**#-**	**0.04 mg/L 25 °C**	**-**	**6.51**	**$94**	**Hydrocarbon (Halogenated)**	**Pesticide Intermediate**
Kepone	**143-50-0**	**490.6**	**Y**	**#-**	**2.70 mg/L @ 25 °C**	**-**	**5.41**	**$123**	**Hydrocarbon (Halogenated)**	**Pesticide**
Progesterone	**57-83-0**	**314.5**	**Y**	**#-**	**8.8 mg/L @ 25 ˚C**	**-**	**3.87**	**$25**	**Steroid**	**Pharmaceutical, Veterinary Agent**
Bisphenol B	77-40-7	242.3	Y	#	1 g in 50 mL	-	2.30	$110	Phenol	Chemical Intermediate, Flame Retardant, Fungicide
o,p'* –DDT	**789-02-6**	**354.5**	**Y**	**#**	**0.085 mg/L @ 25˚ C**	**-**	**6.79**	**$714**	**Hydrocarbon (Halogenated)**	**Pesticide**
Diethylstilbestrol	**56-53-1**	**268.4**	**Y**	**#**	**12 mg/L @ 25˚ C**	**-**	**5.07**	**$47**	**Hydrocarbon (Cyclic)**	**Pharmaceutical, Veterinary Agent**
Fluoranthene	**206-44-0**	**202.3**	**Y**	**#**	**0.2 mg/L**	**soluble**	**5.16**	**$21**	**Polycyclic Compound**	**Industrial Chemical, Laboratory Chemical, Pharmaceutical Intermediate**

Substance	CASRN[a]	MW[b]	EPA Reference Chemicals	AR Antagonism[c]	Solubility in Water[d]	Solubility in DMSO	log K_{ow}[d]	Total Cost Per 500mg[e,f]	MESH Chemical Class[g]	Product Class
Flutamide	13311-84-7	276.2	Y	#	9.45 mg/L @ 25 °C	soluble	3.35	$22	Amide	Pharmaceutical, Veterinary Agent
Genistein	446-72-0	270.2	Y	#	insoluble	50 mg/ml @ 25° C	2.84	$943	Flavonoid, Heterocyclic Compound	Natural Product, Pharmaceutical
Linuron	**330-55-2**	**249.1**	**Y**	**#**	**75 mg/L @ 25 °C**	**-**	**3.2**	**$124**	**Urea**	**Herbicide**
***p,p'*- Methoxychlor**	**72-43-5**	**345.7**	**Y**	**#**	**30.5 mg/L @ 25 °C**	**-**	**5.08**	**$17**	**Hydrocarbon (Halogenated)**	**Pesticide, Veterinary Agent**
Procymidone	**32809-16-8**	**284.1**	**Y**	**#**	**4.5 mg/L @ 25 °C**	**-**	**-**	**$110**	**Polycyclic Compound**	**Fungicide**
Resveratrol	501-36-0	228.2	N	#	-	-	3.08	$226	Hydrocarbon (Cyclic)	Natural Product
Atrazine	**1912-24-9**	**215.7**	**Y**	**-**	**34.7 mg/L @ 26° C**	**183 g/kg @ 25° C**	**2.61**	**$68**	**Heterocyclic Compound**	**Herbicide**
Finasteride	**98319-26-7**	**372.5**	**Y**	**-**	**-**	**-**	**-**	**$377**	**Steroid**	**Pharmaceutical**
Fluoxymestrone	**76-43-7**	**336.4**	**Y**	**-**	**practically insoluble**	**-**	**2.38**	**$131**	**Steroid**	**Pharmaceutical**

Substance	CASRN[a]	MW[b]	EPA Reference Chemicals	AR Antagonism[c]	Solubility in Water[d]	Solubility in DMSO	log K_{ow}[d]	Total Cost Per 500mg[e,f]	MESH Chemical Class[g]	Product Class
Ketoconazole	**65277-42-1**	**531.4**	**Y**	**-**	**0.087 mg/L @ 25 °C**	**-**	**4.35**	**$380**	**Heterocyclic Compound**	**Pharmaceutical**
Butylbenzyl phthalate	85-68-7	312.4	Y	-	2.69 mg/L @ 25˚ C	-	4.91	$79	Carboxylic Acid, Phthalic Acid	Chemical Intermediate, Plasticizer
Testosterone	**58-22-0**	**288.4**	**N**	**-**	**practically insoluble**	**soluble**	**-**	**$26**	**Steroid**	**Pharmaceutical, Veterinary Agent**
Actinomycin D	**50-76-0**	**1255.4**	**Y**	**n.d.[1]**	**1 g/L at 37 ˚C**	**10 mg/ml**	**3.21 @ pH of 7.4**	**$2,285**	**Heterocyclic Compound, Polycyclic Compound**	**Laboratory Chemical, Pharmaceutical, Veterinary Agent**
Ammonium perchlorate	7790-98-9	117.5	N	n.d.	200 g/L at 25 ˚C	-	-	$55	Amine, Onium Compound	Industrial Chemical, Laboratory Chemical, Pharmaceutical
4-Androstenedione	**63-05-8**	**286.4**	**N**	**n.d.**	**57.8 mg/mL 25 °C**	**-**	**2.75**	**$53**	**Steroid**	**Pharmaceutical**
4-Hydroxy androstenedione	566-48-3	302.4	Y	n.d.	-	-	-	$107	Steroid	Pharmaceutical

Substance	CASRN[a]	MW[b]	EPA Reference Chemicals	AR Antagonism[c]	Solubility in Water[d]	Solubility in DMSO	log K_{ow}[d]	Total Cost Per 500mg[e,f]	MESH Chemical Class[g]	Product Class
Apigenin	520-36-5	270.2	N	n.d.	183 mg/L @ 25˚ C	27 mg/ml @ 25˚ C	-	$790	Heterocyclic Compound	Dye, Natural Product, Pharmaceutical Intermediate
Apomorphine	58-00-4	267.3	Y	n.d.	1.660 g/mL	-	2.3	$428	Heterocyclic Compound	Pharmaceutical, Veterinary Agent
2-*sec*-Butylphenol	89-72-5	150.2	N	n.d.	insoluble	-	3.27	$16	Phenol	Chemical Intermediate, Pesticide Intermediate, Plasticizer
Chrysin	480-40-0	254.24	Y	n.d.	84 mg/L @ 25 °C	-	3.52	$60	Flavonoid, Heterocyclic Compound	Natural Product
Clomiphene citrate	50-41-9	598.1	Y	n.d.	slightly soluble	-	-	$45	Amine, Carboxylic Acid, Heterocyclic Compound	Pharmaceutical
Corticosterone	**50-22-6**	**346.5**	**Y**	**n.d.**	**199 mg/L @ 25˚ C**	-	-	**$47**	**Steroid**	**Pharmaceutical**
Coumestrol	479-13-0	268.2	Y	n.d.	practically insoluble	-	-	$1,550	Heterocyclic Compound	Natural Product
4-Cumylphenol	599-64-4	212.3	N	n.d.	insoluble	-	-	$24	Phenol	Chemical Intermediate
Cycloheximide	66-81-9	281.4	N	n.d.	0.00021 mg/L @ 25 ˚C	-	0.55	$45	Heterocyclic Compound	Fungicide, Pharmaceutical, Veterinary Agent

Substance	CASRN[a]	MW[b]	EPA Reference Chemicals	AR Antagonism[c]	Solubility in Water[d]	Solubility in DMSO	log K_{ow}[d]	Total Cost Per 500mg[e,f]	MESH Chemical Class[g]	Product Class
Daidzein	486-66-8	254.2	Y	n.d.	practically insoluble	10 mg/ml	-	$735	Flavonoid, Heterocyclic Compound	Natural Product
Dexamethasone	**50-02-2**	**392.5**	**Y**	**n.d.**	**10 mg/100mL @ 25 °C**	**-**	**-**	**$352**	**Steroid**	**Pharmaceutical, Veterinary Agent**
Dibenzo[*a,h*]anthracene	53-70-3	278.4	Y	n.d.	practically insoluble	-	6.5	$352	Polycyclic Compound	Laboratory Chemical, Natural Product
Dicofol	**115-32-2**	**370.489**	**Y**	**n.d.**	**1.2 mg/L @24°C**	**-**	**4.28**	**$88**	**Hydrocarbon (Cyclic), Hydrocarbon (Halogenated)**	**Pesticide**
5α-Dihydrotestosterone	**521-18-6**	**290.4**	**Y**	**n.d.**	**practically insoluble**	**-**	**3.55**	**$27**	**Steroid**	**Pharmaceutical**
17α-Estradiol	57-91-0	272.4	Y	n.d.	3.9 mg/L	-	3.94	$230	Steroid	Pharmaceutical, Veterinary Agent
17α-Ethinyl estradiol	**57-63-6**	**296.4**	**Y**	**n.d.**	**insoluble**	**-**	**3.67**	**$35**	**Steroid**	**Pharmaceutical, Veterinary Agent**
17ß-Estradiol	**50-28-2**	**272.4**	**Y**	**n.d.**	**3.60 mg/L @ 27 °C**	**soluble**	**4.01**	**$151**	**Steroid**	**Pharmaceutical, Veterinary Agent**
Estrone	**53-16-7**	**270.4**	**Y**	**n.d.**	**0.003 g/ 100 mL @ 25˚ C**	**-**	**3.13**	**$14**	**Steroid**	**Pharmaceutical, Veterinary Agent**
Fenarimol	60168-88-9	331.2	Y	n.d.	14 mg/L @ 25˚ C	-	3.6	$375	Heterocyclic Compound, Pyrimidine	Fungicide

Substance	CASRN[a]	MW[b]	EPA Reference Chemicals	AR Antagonism[c]	Solubility in Water[d]	Solubility in DMSO	log K_{ow}[d]	Total Cost Per 500mg[e,f]	MESH Chemical Class[g]	Product Class
Flavone	525-82-6	222.2	N	n.d.	0.1 mg/L @ 25˚ C	-	5.08	$48	Flavonoid, Heterocyclic Compound	Natural Product, Pharmaceutical
Haloperidol	52-86-8	375.9	Y	n.d.	1.4 mg/L @ 25 °C	soluble	4.3	$54	Ketone	Pharmaceutical, Veterinary Agent
meso-Hexestrol	84-16-2	270.4	Y	n.d.	-	-	-	$35	Steroid	Pharmaceutical, Veterinary Agent
4-Hydroxytamoxifen	**68047-06-3**	**387.5**	**N**	**n.d.**	**practically insoluble**	**soluble**	**-**	**$6,090**	**Hydrocarbon (Cyclic)**	**Pharmaceutical**
Kaempferol	520-18-3	286.2	Y	n.d.	slightly soluble	-	1.96	$390	Flavonoid, Heterocyclic Compound	Natural Product
Medroxyprogesterone acetate	**71-58-9**	**386.5**	**Y**	**n.d.**	**practically insoluble**	**-**	**-**	**$105**	**Steroid**	**Pharmaceutical**
Morin	480-16-0	302.2	Y	n.d.	250 mg/L @ 25 °C	-	1.54	$14	Flavonoid, Heterocyclic Compound	Dye, Natural Product, Pharmaceutical Intermediate
Norethynodrel	68-23-5	298.4	Y	n.d.	practically insoluble	-	3.51	$78	Steroid	Pharmaceutical
19-Nortestosterone	**434-22-0**	**274.4**	**N**	**n.d.**	**-**	**-**	**-**	**$90**	**Steroid**	**Pharmaceutical, Veterinary Agent**
4-*tert*-Octylphenol	**140-66-9**	**206.3**	**Y**	**n.d.**	**-**	**-**	**-**	**$28**	**Phenol**	**Chemical Intermediate, Pharmaceutical Intermediate**
Oxazepam	604-75-1	286.7	Y	n.d.	179 mg/L	-	2.24	$463	Heterocyclic Compound	Pharmaceutical, Veterinary Agent

Substance	CASRN[a]	MW[b]	EPA Reference Chemicals	AR Antagonism[c]	Solubility in Water[d]	Solubility in DMSO	log K_{ow}[d]	Total Cost Per 500mg[e,f]	MESH Chemical Class[g]	Product Class
Ethyl paraben	120-47-8	166.2	Y	n.d.	885 mg/L @ 25 °C	-	2.47	$8	Carboxylic Acid, Phenol	Pharmaceutical, Preservative
Phenobarbital	**50-06-6**	**232.2**	**Y**	**n.d.**	**1300 mg/L @ 25 °C**	**-**	**1.47**	**$56**	**Heterocyclic Compound, Pyrimidine**	**Pharmaceutical, Veterinary Agent**
Phenolphthalin	81-90-3	320.3	Y	n.d.	175 mg/L @ 20 °C	-	3.95	$26	Carboxylic Acid, Phenol	Dye, Laboratory Chemical
Diethylhexyl phthalate	**117-81-7**	**330.2**	**Y**	**n.d.**	**0.285 mg/L @ 24 °C**	**-**	**7.6**	**$26**	**Phthalic Acid**	**Pesticide Intermediate, Plasticizer**
Di - *n* -butyl phthalate	**84-74-2**	**278.3**	**Y**	**n.d.**	**-**	**-**	**-**	**$34**	**Ester, Phthalic Acid**	**Cosmetic Ingredient, Industrial Chemical, Plasticizer**
Pimozide	2062-78-4	461.6	Y	n.d.	insoluble	18 mg/mL	-	$45	Heterocyclic Compound	Pharmaceutical
Propylthiouracil	51-52-5	170.2	Y	n.d.	1204 mg/L @ 25 °C	-	-	$28	Heterocyclic Compound, Pyrimidine	Pharmaceutical, Veterinary Agent
Raloxifene[i]	82640-04-8	510.1	N	n.d.	insoluble	28 mg/ml	-	$235	Hydrocarbon (Cyclic)	Pharmaceutical
Reserpine	50-55-5	608.7	Y	n.d.	73 mg/L @ 30 °C	-	-	$76	Heterocyclic Compound, Indole	Pharmaceutical, Veterinary Agent
Sodium azide	**26628-22-8**	**65.0**	**Y**	**n.d.**	**41 g/100 mL @ 15 °C**	**-**	**-**	**$17**	**Azide, Salt (inorganic)**	**Chemical Intermediate, Fungicide, Herbicide**

Substance	CASRN[a]	MW[b]	EPA Reference Chemicals	AR Antagonism[c]	Solubility in Water[d]	Solubility in DMSO	log K_{ow}[d]	Total Cost Per 500mg[e,f]	MESH Chemical Class[g]	Product Class
Tamoxifen	10540-29-1	371.5	Y	n.d.	practically insoluble	soluble	-	$125	Hydrocarbon (Cyclic)	Pharmaceutical
Methyl testosterone	**58-18-4**	**302.5**	**Y**	**n.d.**	**-**	**-**	**3.32**	**$26**	**Steroid**	**Pharmaceutical, Veterinary Agent**
12-*O*-Tetradecanoylphorbol-13-acetate	**16561-29-8**	**616.8**	**-**	**n.d.**	**-**	**soluble**	**-**	**$11,200**	**Hydrocarbon (Cyclic)**	**Laboratory Chemical**
L-Thyroxine	51-48-9	776.9	Y	n.d.	slightly soluble	-	-	$65	Amino Acid	Pharmaceutical, Veterinary Agent
17b-Trenbolone	**10161-33-8**	**270.4**	**Y**	**n.d.**	**-**	**-**	**-**	**$130**	**Steroid**	**Pharmaceutical**
2,4,5-Trichlorophenoxyacetic acid	**93-76-5**	**255.5**	**Y**	**n.d.**	**278 mg/L**	**-**	**3.31**	**$48**	**Carboxylic Acid**	**Herbicide**

Substances listed in bolded text are included on the ICCVAM Minimum List of Substances for Validation of AR binding and TA Assays.
**p,p'*-DDE =1,1-Dichloro-2,2-di(*p*-chlorophenyl)ethylene; *o,p'*-DDT =1,1,1-Trichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)ethane
[a] CASRN = Chemical Abstracts Service Registry Number
[b] MW = Molecular Weight
[c] ### Indicates that the substance was uniformly positive in multiple assays; ## indicates that the substance was positive in the majority of assays in which it was tested; # indicates that the substance was positive in the single assay in which it was tested; #- indicates the substance was positive in one assay but was also negative in one or more assays; - indicates that the substance was uniformly negative in multiple assays.
[d] Information on Solubility in Water, Solubility in DMSO, and log $K_{ow,}$ were obtained from the National Library of Medicine's ChemIDplus http://chem.sis.nlm.nih.gov/chemidplus/, and from manufacturer Materials Safety Data Sheets (MSDSs).
[e] 500 mg is the expected minimum amount of substance required per laboratory to conduct an endocrine disruptor (ED) validation study.
[f] Pricing information was obtained from vendors during October of 2005 and reflects the cost of 500 mg of substance, or the minimum amount sold.
[g] MeSH = Medical Subject Headings, information on chemical class criteria can be obtained at www.nlm.nih.gov/MeSH
[h] A "-" in the fields for Solubility in Water, Solubility in DMSO or low K_{ow} indicates that there is no data for this field.
[i] Raloxifene may act as an agonist in some *in vitro* systems.
[j] Y indicates that this substance is included on the EPA reference chemical list.
[k] N indicates that this substance is not included on the EPA reference chemical list.
[l] n.d. indicates that no relevant data were identified.